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Review Article on Lung Cancer and The Immune System

# Thermal ablation in non-small cell lung cancer: a review of treatment modalities and the evidence for combination with immune checkpoint inhibitors

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**Abstract:** Lung cancer is the leading cause of cancer death worldwide, with approximately 1.6 million cancer related deaths each year. Prognosis is best in patients with early stage disease, though even then five-year survival is only 55% in some groups. Median survival for advanced non-small cell lung cancer (NSCLC) is 8–12 months with conventional treatment. Immune checkpoint inhibitor (ICI) therapy has revolutionised the treatment of NSCLC with significant long-term improvements in survival demonstrated in some patients with advanced NSCLC. However, only a small proportion of patients respond to ICI, suggesting the need for further techniques to harness the potential of ICI therapy. Thermal ablation utilizes the extremes of temperature to cause tumour destruction. Commonly used modalities are radiofrequency ablation (RFA), cryoablation and microwave ablation (MWA). At present thermal ablation is reserved for curative-intent therapy in patients with localized NSCLC who are unable to undergo surgical resection or stereotactic ablative body radiotherapy (SABR). Limited evidence suggests that thermal ablative modalities can upregulate an anticancer immune response in NSCLC. It is postulated that thermal ablation can increase tumour antigen release, which would initiate and upregulated steps in the cancer immunity cycle required to elicit an anticancer immune response. This article will review the current thermal ablative techniques and their ability to modulate an anti-cancer immune response with a view of using thermal ablation in conjunction with ICI therapy.

**Keywords:** Non-small cell lung cancer (NSCLC); cancer immunity; immunotherapy; thermal ablation

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Lung cancer is the leading cause of cancer death worldwide. More than 80% of lung cancers are non-small cell lung cancer (NSCLC) and patients with early stage disease have the best outcomes. However, most NSCLC are diagnosed at an advanced stage where the median survival is 12 months with conventional treatment of chemotherapy and radiotherapy (1). The recent development of immune checkpoint inhibitor (ICI) therapy (immunotherapy)

has significantly improved survival in some patients. Unfortunately, not all patients respond to immunotherapy and others who may respond initially can go on to develop resistance (2,3). New approaches are required to fully utilise the benefits of immunotherapy. In this article we review the use of thermal ablation modalities in NSCLC and their potential to enhance natural and immunotherapy anti-tumour responses.

## Thermal ablation modalities

Thermal ablation uses extreme temperatures to induce tissue damage and is used to treat several malignancies including liver, kidney and lung, as an alternative for curative-intent therapy in medically inoperable patients with Stage I NSCLC (4,5).

The commonly used thermal ablation techniques are radiofrequency ablation (RFA), microwave ablation (MWA) and cryoablation. The mechanism by which each modality induces tumour destruction has been described extensively in the literature (6-9). Ablative modalities are delivered percutaneously directly into the tumour in current clinical practice, but bronchoscopic ablative techniques are in development (10,11).

Cryoablation is the rapid cooling of tissue to a temperature low enough that it results in tissue damage. Cryoablation probes achieve this by harnessing the Joule-Thompson effect where a drop in temperature occurs as a result for the rapid expansion of certain liquids to gas such as argon or nitrogen. Temperatures as low as –160 ℃ can be achieved in tissue with cryoablation, resulting in ice crystal formation with cell death caused by cell membrane rupture, cell desiccation and osmotic shock (7).

RFA uses the heat energy that is created by high frequency alternating currents to cause tissue damage and coagulative necrosis. Tissue damage is dependent on the electrical conductance of tissue. Low conductance of lung, and close proximity to large blood vessels and airways can reduce the efficacy of RFA (8,9).

MWA uses the heat generated by electromagnetic waves between frequencies of 900–2,500 MHz to cause cell death. Electromagnetic energy is less dependent on tissue characteristics than RFA and can lead to more accurate and larger ablation zones (6).

## Clinical outcomes of thermal ablation

Thermal ablation is reserved for patients with inoperable localized NSCLC. Overall survival benefit between thermal ablation and stereotactic ablative body radiotherapy (SABR) appears comparable in a few large retrospective studies (12-14). The ability to achieve complete ablation, and the progression free survival with RFA and MWA have been found to be comparable, particularly for those with tumours less than two centimetres in diameter (14-20). Five-year overall survival rates of 27–67% have been reported with cryoablation, RFA and MWA (21-23). This is comparable to SABR, where five-year survival is approximately 30% and is inferior to five-year survival rates of those who undergo surgical resection were rates are 48–65% depending on type of surgery and degree of lymph node involvement (24).

The complications of thermal ablation techniques relate to route of delivery (percutaneous or bronchoscopic) and to consequences of tissue ablation. Pneumothorax rates of 30–40% are described with the percutaneous approach (14,23,25,26). Of these about 13% will require chest tube insertion (14,23,25). Complications related to the consequences of tissue ablation include pleural effusion (5.2–9.6%), haemoptysis (3.9%), pneumonia (5.7%), respiratory failure (3.5%) and lung collapse (4%) (26). Clinically significant haemorrhage is rare. In the same way that bronchoscopic techniques have proven far safer than percutaneous biopsy for diagnosis of peripheral pulmonary lesions (27), bronchoscopic ablative modalities are associated with a superior safety profile, with initial studies demonstrating minimal adverse events (28-32).

## Cancer and the immune system

The revolutionary improvements in lung cancer treatment with the use of immunotherapy has highlighted the need for a deeper understanding of the role of the immune system in the evolution of cancer (1,33).

Oncogenesis requires multiple events that enable tumour cell survival, including multiple somatic mutations that activate oncogenic drivers or delete tumour suppressors. The accumulation of mutations can result in cancer cells that are genetically diverse from the patient and as such should be identified as foreign by the host immune system, resulting in an anti-cancer immune response.

The cancer-immunity cycle illustrates the steps that are required to enable an anti-cancer immune response (*Figure 1*). Cancer antigens are presented via antigen presenting cells such as dendritic cells to T cells causing activation and priming of the immune response. This leads to trafficking and infiltration of cytotoxic T cells

 

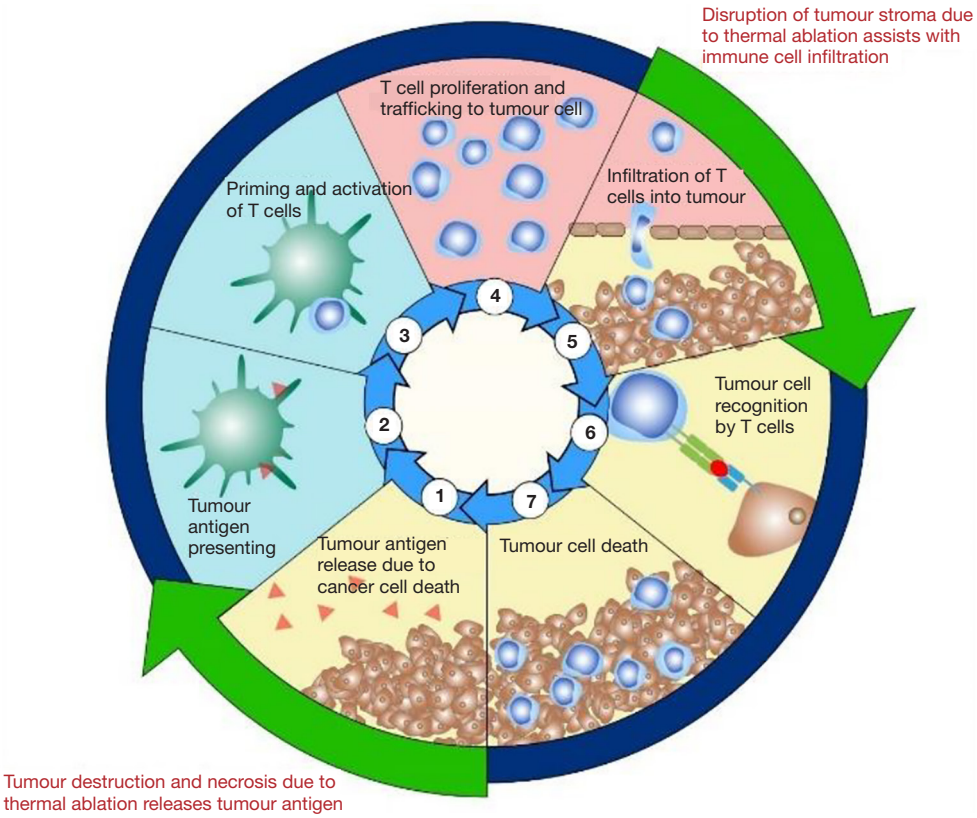


**Figure 1** The cancer immunity cycle and the effects of thermal ablation: the cancer immunity cycle demonstrates the steps needed to induce an anticancer immune response. Step 1: tumour antigen release; step 2: Antigen presenting by dendritic cells; step 3: Priming and activation of T cells; step 4: T cell proliferation and trafficking to tumour cells; step 5: infiltration of T cells into tumour; step 6: tumour cell recognition by T cells and step 7: Tumour cell death. Immune checkpoint inhibitors can assist with steps 3, 6 and 7. Current evidence would suggest that thermal ablation would upregulate steps 1 and 2 as well as augment step 5.

into the tumour resulting in cancer cell death and further antigen release (34). A hallmark of tumour development is immunoevasion (35), which often occurs through upregulation of negative regulatory pathways (checkpoints) of immune homeostasis. Two examples are cytotoxic T-lymphocyte protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1). CTLA-4 limits T cell activation by directly competing with the ligands of the co-stimulatory protein CD28, CD80 and CD86 (36). PD-1 is a cell surface receptor expressed on T-cells. Binding to its ligand program cell death ligand 1 (PD-L1) (*Figure 2*), results in activation of inhibitory signal pathways that lead to T-cell attenuation and exhaustion (37-41). Durvalumab, Pembrolizumab and nivolumab are anti-PD-L1 monoclonal antibodies that are currently used in current clinical practise. Ipilimumab is an anti-CTLA-1 monoclonal antibody that has recently been approved for use in NSCLC. However, despite the excellent response to ICI therapy in some patients with durable responses that can last many years, this only appears to occur in about 20% of those that are treated (2). Some patients with initial respond to PD-1 inhibitors go on to develop resistance as well (3).

Response to ICI therapy appears to correlate with types of T-cell immune response. Several studies that have assessed histologic samples prior to initiation of therapy have demonstrated several immune phenotypes that predict response to immunotherapy. These profiles are immune-inflamed, immune excluded and immune-desert phenotypes. The immune inflamed phenotype is characterised by a tumour microenvironment where immune cells (especially CD4+ and CD8+ T cells) and cancer cells are in close proximity within the tumour parenchyma. These tumours are also associated with elevated levels of pro-inflammatory cytokines that promote T cell activation and expansion.

 

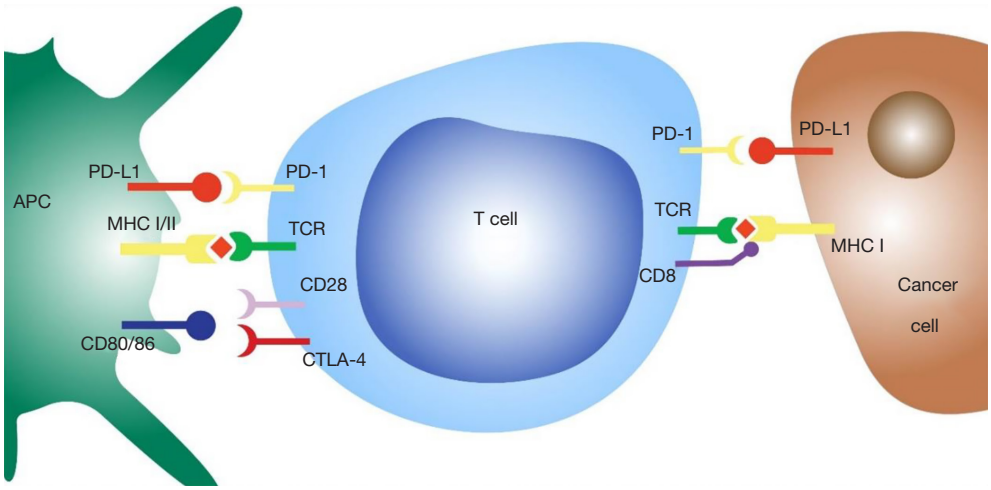


**Figure 2** T cell interaction with antigen presenting cells and tumour cells. APC, antigen presenting cell; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; MHC, major histocompatibility complex; CTLA4, cytotoxic T lymphocyte protein 4; TCR, T cell receptor.

The immune-excluded phenotype demonstrates abundant immune cells; however, these cells are found in the stroma and do not penetrate the tumour parenchyma. The immune-desert phenotype shows a paucity of T cells in both the stroma and parenchyma of the tumour (42,43).

Immune-inflamed tumours have higher response rates to anti-PD-L1 and anti-PD-1 therapy. However, not all immune-inflamed tumours respond to immunotherapy, highlighting that immune cell infiltration on its own is not sufficient to induce an immune response and that implies cancer immunity is affected by tumour, host and environmental factors. These include intrinsic tumour properties such as cytokine release (44) and genetic composition (44) [including tumour mutational burden (TMB)]; and extrinsic factors such as the gut microbiome (45-49), the presence of infection (44) and the exposure to sunlight (50). These factors promote and suppress cancer immunity and sit in an equilibrium that is defined as the cancer-immune set point. This threshold needs to be surpassed for an individual with cancer to respond to immunotherapy (44).

## Thermal ablation and the immune system

Recent pre-clinical work and some clinical studies have suggested that percutaneous thermal ablative therapies may alter the immune-profile of patients with cancer by activating various steps in the cancer immunity cycle. This would imply that thermal ablation has the potential to improve the efficacy of immunotherapy (51,52). We will explore further the current evidence that supports this for RFA, cryoablation and MWA.

Though evidence is limited in NSCLC, immunogenic changes with thermal ablation have been assessed in several malignancies, especially liver cancer, breast cancer, renal cell cancer and prostate cancer. Early studies in both cryoablation and RFA demonstrated tumour regression of untreated metastatic lesions (abscopal effect) with associated upregulation of immune cells in peripheral blood (53-59).

Tumour necrosis occurs due to thermal ablation and results in neoantigen release from the tumour. RFA has been shown to increase carcinoembryonic antigen (CEA) levels in liver metastasis of colorectal cancer and similar changes in serum prostate specific antigen (PSA) in prostate cancer has been seen with cryoablation (60,61). Along with an increase in neoantigen release, an upregulation of danger signals is seen and together this assists in modulating the first step of the cancer immunity cycle. Danger signals are endogenous molecules that are released by damaged cells and have various effects on cancer immunity. Heat shock protein 70 (HSP70) is a danger signal that has been found to be elevated post cryoablation and RFA in melanoma (62). Heat-shock proteins assists in antigen presenting, by both chaperoning antigen to dendritic cells and by upregulating MHC class 1 expression (63,64). This induction of neoantigen expression and danger signal release leads

 

to increased neoantigen presentation as evidenced by the increased numbers of dendritic cells and enhanced dendritic cell maturation that has been observed with both cryoablation and RFA in several cancers including NSCLC (65,66).

Evidence of T cell trafficking to ablated tumour as a result of increased neoantigen presentation has been seen in preclinical studies. Chemokine ligand 21 (CCL21) and intercellular adhesion molecule 1 (ICAM-1) are key molecules associated with T cell trafficking. The expression of CCL21 and ICAM-1 have been shown to be overexpressed in endothelial venules of the tumour draining lymph node and in CD31 positive endothelial cells of the tumour post RFA. As a result, ablation creates a localised T cell homing function causing an increase in naïve CD8+ T cells in ablated tumour tissue and tumour draining lymph nodes. The increase in T cell trafficking can be seen as early as 1 hour post ablation in mouse studies and appears to peak somewhere between 6 to 12 hours post ablation (67) leading to an increase in tumour infiltrating lymphocytes.

Preclinical and clinical studies have shown that the lymphocytes that infiltrate the tumour as a result of thermal ablation are predominantly CD4+ and CD8+ T cells (54,56,66,68-77). This response appears more robust with RFA and cryoablation than MWA (72,73,78). Upregulation of PD-L1 expression in resected tumour and PD1 expression in tumour infiltrating CD8+ and CD4+ lymphocytes have been seen post RFA in colorectal cancer (74). Natural killer (NK) cell and macrophage infiltration also appears to increase with thermal ablation (71,73).

This increase in tumour infiltrating lymphocytes has been associated with elevated interferon-γ (IFN-γ) levels post ablation, implying an upregulation of a T helper 1 (Th1) response that in turn would correspond with a cell mediated immune response (79). A Th1 response has also been observed in patients with liver cancer who demonstrate an abscopal effect with thermal ablation. Furthermore, the degree of the Th1 response appears to correlate with the observed clinical response in prostate cancer and renal cell carcinoma (80,81). Additionally, the pro-inflammatory cytokines IL-1β, IL-6 and IL-8 have been noted to be elevated post thermal ablation (54,82,83). These cytokines have been shown to assist with T cell proliferation and trafficking (84); as well promoting a Th1 response (85).

Importantly, along with these changes in T (CD4+ and CD8+) effector cells, a reduction of immunosuppressive regulatory T cells (Treg) post ablation has been noted in studies of renal cell cancer, hepatocellular cancer (HCC) and prostate cancer (54,77,86-88). A higher number of CD4+ and CD8+ T cells and a lower number of Treg cells and myeloid-derived suppressor cells (MDSC) post thermal ablation have been shown to have a positive effect on tumour progression and survival (86,89).

While most of the evidence supports a robust anti-cancer immune response with thermal ablation it only seems to be transient. The immune effect appears to last about 4 weeks, as described in a preclinical study where immune cells taken 4 and 8 weeks post ablation underwent a tumour rechallenge and anti-tumour cytolytic effect was only seen with immune cells from 4 weeks post ablation (90). In human studies, tumour antigen specific CD4+ and CD8+ T cells have been seen to persist in the peripheral blood between 2–4 weeks post ablation (54,76,77).

## Thermal ablation and immunotherapy

While the anti-cancer immune response post ablation appears transient, there is robust evidence that it upregulates various steps in the cancer immunity cycle and could act to enhance the effect of immunotherapies. This has been assessed in some preclinical and clinical studies (*Table 1*).

Several types of immunotherapy have been assessed in combination with thermal ablation. These include dendritic cell injection; the use of the immune adjuvant CPG-oligodeoxynucleotides (CpG-ODN); injection of OK432 activated dendritic cells; IL-2 injection and granulocyte macrophage colony stimulating factor (GM-CSF) injection. All therapies in conjunction with thermal ablation appeared to promote a stronger immune response than either ablation or immunotherapy alone (*Table 1*) (62,69,71,74,81,91-110).

More importantly, a few studies have combined ICI therapy with thermal ablation in both preclinical and clinical settings. Similar to other immunotherapies combination, the addition of ICI to thermal ablation appeared to produce a more robust response compared to either ICI or ablation alone (*Table 1*) (74,92,97,100,105,106,109).

One pilot study by McArthur *et al.* assessed patients with breast cancer who were treated with combined cryoablation and a CTLA-4 antagonist prior to mastectomy. Combination treatment was observed to be safe in this cohort. Combination treatment demonstrated a more sustained immune response than either ICI or thermal ablation alone. In peripheral blood this was characterised by an increase in Th1 cytokines (IFN-γ) and activated (ICOS+) and proliferating (Ki67+) CD4+T

 

**Table 1** Studies where thermal ablation is combined with immunotherapy

| Study | Ablation modality | Tumour | Immunotherapy | Clinical/preclinical | Outcome |
|---|---|---|---|---|---|
| Liang *et al.* (91) | Cryoablation | Breast cancer | NK cell therapy; Herceptin | Human, n=48 | • Well tolerated<br>• Improved progression free survival |
| McArthur *et al.* (92) | Cryoablation | Breast cancer | Anti-CTLA4-antibody | Human, n=19 (treat and resect study) | • Safe and well tolerated<br>• Increase Th1 cytokines, CD4+ and CD8+ T cells in peripheral blood<br>• T-eff: T-reg cell ratio increased in tissue |
| Si *et al.* (93) | Cryoablation | Prostate cancer | GM-CSF | Human, n=12 | • Improved tumour specific T cell response |
| Yuangying *et al.* (94) | Cryoablation | NSCLC | DC-CIK injection | Human, retrospective study, n=161 | • Improved survival |
| Thakkur *et al.* (81) | Cryoablation | Renal cell cancer | GM-CSF | Human, n=6 | • Increase in Th1 cytokines with increase in tumour specific T cells |
| Niu *et al.* (95) | Cryoablation | HCC | DC-CIK | Human, n=21 | • Improved survival |
| Niu *et al.* (96) | Cryoablation | Prostate cancer | DC-CIK | Human, n=31 | • Improved survival |
| Domingo-Musibay *et al.* (62) | RFA; Cryoablation | Melanoma | GM-CSF | Human, n=9 | • Combination was safe and well tolerated |
| Benzon *et al.* (97) | Cryoablation | Prostate cancer | Anti-CTLA4-antibody Anti-PD1- antibody | Mouse | • Improved survival<br>• Increase in CD3+ and CD8+ T cells |
| Zhang *et al.* (98) | Cryoablation | Lewis lung cancer | DC+CpG-ODN injection | Mouse | • Improved survival |
| Lin *et al.* (99) | Cryoablation | Glioma | DC injection | Mouse | • Improved survival<br>• Increase CD3+, CD4+ T cells |
| Li *et al.* (100) | Cryoablation | Prostate cancer | Anti-CTLA4-antibody | Mouse | • Reduced number of T reg cells |
| Alteber *et al.* (101) | Cryoablation | Lewis lung cancer | DC + CpG-ODN | Mouse | • Reduced tumour and metastasis growth<br>• Resistant to rechallenge |
| Machlenkin *et al.* (102) | Cryoablation | Lung cancer; Melanoma | DC injection | Mouse | • Improved overall survival<br>• Reduction in metastasis<br>• Increase Th1 response |
| Xu *et al.* (103) | Cryoablation | Glioma | GM-CSF | Mouse | • Increase in activated DC<br>• Increase Th1 response<br>• Increase in tumour specific T cells |

**Table 1** (*continued*)

**Table 1** (*continued*)

| Study | Ablation modality | Tumour | Immunotherapy | Clinical/preclinical | Outcome |
|---|---|---|---|---|---|
| den Brok *et al.* (104) | Cryoablation | Melanoma | CpG-ODN | Mouse | • Increase anti-tumour immunes response<br>• Increase in DC function |
| Waitz *et al.* (105) | Cryoablation | Prostate | Anti-CTLA4 antibody | Mouse | • Improved progression free survival<br>• Resistance to tumour rechallenge<br>• Associated higher T-eff: T-reg cell ratio |
| den Brok *et al.* (106) | Cryoablation; RFA | Melanoma | Anti-CD25 antibody; anti-CTLA4 antibody | Mouse | • Improved survival<br>• Resistance to tumour rechallenge |
| Shi *et al.* (74) | RFA | Colorectal cancer | Anti-PD1 antibody | Mouse | • Improved survival and reduction in distant metastasis<br>• Associated higher T-eff: T-reg cell ratio |
| Nakagawa *et al.* (69) | RFA | Colon cancer | Activated DC injection | Mouse | • Reduction in distant metastasis |
| Kroeze *et al.* (71) | RFA | Renal cell carcinoma | IL-2 | Mouse | • Reduction in distant metastasis<br>• Higher levels of CD4+ and CD8+ T cell in tumour tissue |
| Hamamoto *et al.* (107) | RFA | VX2 | OK-432 | Rabbit | • Improved survival<br>• Reduce growth of distant tumour |
| Habibi *et al.* (108) | RFA | Breast cancer | IL-7; IL-5 | Mouse | • Reduce tumour growth and metastasis<br>• Reduced MDSC |
| Zhu *et al.* (109) | MWA | Breast cancer | Combination ICI | Mouse | • Longer survival<br>• Resistance to tumour rechallenge<br>• Associated higher levels of IFNγ |
| Kuang *et al.* (110) | MWA | Lewis lung | IL2; GMCSF | Mouse | • Longer survival |

NK, natural killer cells; DC, dendritic cells; GM-CSF, granulocyte macrophage colony stimulating factor; DC-CIK, dendritic cell and cytokine induced killer cells; CPG-ODN, CPG-oligodeoxynucleotides; CTLA4, cytotoxic T lymphocyte associated protein 4; PD1, programmed cell death 1; CD, clusters of differentiation; IL, interleukin; Treg, regulatory T cell; Teff, T effector cell; IFN, interferon; MDSC, myeloid derived suppressor cell.

**Table 2** Studies combining thermal ablation and immunotherapy that are currently registered at clinicaltrials.gov

| Identifier | Title | Primary endpoint |
|---|---|---|
| NCT04339218 (111) | Cryoablation in Combination (or Not) With Pembrolizumab and Pemetrexed-carboplatin in 1st-line Treatment for Patients with Metastatic Lung Adenocarcinoma (CRYOMUNE) | 1-year overall survival |
| NCT04201990 (112) | Cryoablation Combined with Camrelizumab and Apatinib for Multiprimary Lung Cancer (CCA-MPLC) | Safety |
| NCT04102982 (113) | Microwave Ablation in Combination with Camrelizumab Versus Camrelizumab in Metastatic Non-small-cell Lung Cancer (MWA in NSCLC) | Overall survival |
| NCT03769129 (114) | Evaluating the Safety and Efficacy of Pembrolizumab Combined with MWA for Advanced NSCLC | Overall survival |
| NCT03290677 (115) | Study of Core Needle Biopsy and Cryoablation of an Enlarging Tumour in Patients with Metastatic Lung Cancer and Metastatic Melanoma Receiving Post-Progression Immune Checkpoint Inhibitor Therapy | Cumulative incidence of treatment related serious adverse events |

cells and CD8+ T cells (92). Currently further trials are underway to assess the synergy of thermal ablation with ICI in NSCLC (111-115) (*Table 2*).

## Non thermal ablation and the immune system

Irreversible electroporation (IRE) is another ablation technique that exposes cells to electric pulses, which leads to cell wall damage and immunogenic cell death. Though not classified as a thermal ablative technique it has shown upregulation of an immune response in pancreatic cancer. In mouse studies with transplantation of renal carcinoma and pancreatic cancer cell lines, IRE lead to an increase in T-cell infiltration and improvements in progression free survival (116,117). IFN-γ increase has also been observed post IRE in rat models with osteosarcoma, this again was associated with an increase in CD4+ T cells in peripheral blood and tumour (118). A study of 34 patients with locally advanced pancreatic cancer that underwent IRE demonstrated similar findings with an increase in CD4+ T cells, CD8+ T cells, NK cells and a reduction in regulatory T cells. This was associated with an increase in IL2 and a decrease in IL-6 and IL-10 (119). Additionally, IRE in combination with PD1 blockade has been found to increase CD8+ T cell tumour infiltration and improve overall survival in mice with pancreatic ductal adenocarcinoma (120). These results suggest that IRE also has potential to upregulate an anticancer immune response. Currently IRE has shown limited utility when delivered percutaneously to lung cancers (121-123), however flexible IRE catheters have been utilised bronchoscopically for management of airway-based inflammation and may be suitable for bronchoscopic IRE treatment of lung cancer (124).

Tumour antigen release, dendritic cell activation and CD8+ T cell infiltration has also been described with radiotherapy. Preclinical studies where radiotherapy was combined with CTLA-4 blockade and anti-PD1 antibody demonstrated improvement in tumour control and anti-tumour immune effect (125). While the safety of combination ICI and radiotherapy has been established (125), clinical trials assessing the immune changes associated with radiotherapy are still underway (126). At present there are no studies comparing the immune effects of radiotherapy to thermal ablation in NSCLC.

## Could ablation be harmful?

While a majority of studies demonstrate a positive anticancer response the with thermal ablation, a few studies have suggested that RFA may promote tumour recurrence in HCC. Aggressive outgrowth of residual hepatic micro-metastasis had been seen in the surrounding tissue of patients undergoing RFA of HCC (127). A hypoxic microenvironment that occurs in HCC post RFA has been shown to enhance the invasive, chemo-resistant and metastatic abilities of the tumour cells, these changes had been seen in patients that underwent incomplete ablation (128). Perhaps in contrast to lung cancer, a chronic inflammatory state in HCC patients may favour a tumour microenvironment that would enhance tumour growth, as suggested by the Th2 cell dominance pre and post RFA in hepatocellular carcinoma in patients with

 

hepatitis C (129).

The cytokines IL-10 and TGF-β have also been noted to be elevated post thermal ablation (54,70,82). IL-10 and TGF-β are immunosuppressive cytokines that act to inhibit dendritic cell maturation and block co-stimulatory pathways and upregulate T reg differentiation (82,130,131). Similarly, IL-6 which assists with T cell trafficking also has negative effects on anticancer immunity. It plays a role in impairing dendritic cell maturation, increasing MDSC proliferation and assisting in tumour cell proliferation (84,132,133).

A retrospective case control study of patients with colorectal cancer and liver metastasis who underwent RFA found that those with incomplete ablation demonstrated significantly shorter time to new metastasis and reduced overall survival when compared to complete ablation (134). Incomplete tumour ablation could alter the balance of apoptotic cell death versus necrotic cell death. Apoptotic cell death has been found to be less immunogenic and may actually inhibit an immune response (135-137).

The effects of residual tumour were further assessed in mice with colon cancer where It was found that when compared to complete RFA, incomplete RFA increased the amount of new metastatic lesions and tumour growth. Anti-PD-1 treatment did not alter the rate of tumour growth or degree of new metastasis in this cohort. Incomplete RFA was associated with an increase in M2 phenotype macrophages, lower numbers of CD4, CD8 T cells and a higher number of Tregs. Tumour proliferation appears to be driven by residual tumour and an increase in tumour associated macrophages (TAMs) (134).

While similar effects have not be seen in cryoablation and MWA studies, this warrants further assessment.

## The role of ablative immunotherapy

As mentioned previously percutaneous thermal ablation is currently used to treat localized NSCLC patient's ineligible for surgery. Ablative immunotherapy, where thermal ablation is combined with ICI, could potentially expand this treatment modality to a broader patient cohort, including surgical patients, as well as later stage tumours. While ICI has significantly improved the survival in patients with metastatic NSCLC, utilising thermal ablation could further improve both rates of response and duration of efficacy. In addition, thermal ablation may be helpful in patients that develop resistance to ICI by increasing neoantigen release and upregulating the cancer immunity cycle.

The current treatment of early stage NSCLC is surgical resection, however 5-year survival is only 55% in some groups, with a large proportion experiencing disease recurrence (138). The use of neoadjuvant ICI therapy has been encouraging in initial studies with complete pathologic response noted in some cases. However, the efficacy again is still limited and using this in conjunction with thermal ablation could improve efficacy. The utility of neoadjuvant ablative immunotherapy would need to be assessed in robust clinical trials to assess its efficacy when compared to current standard treatment.

Current evidence demonstrates that thermal ablation can elicit a significant immune response that may be able to propagate the cancer immunity cycle. We hypothesise that this can shift tumours with an immune desert or immune-excluded phenotype to a more immune-inflamed phenotype by upregulating steps 1 and 2 of the cancer immunity cycle (*Figure 1*). Furthermore, the process of ablation has the ability to directly disrupt the tumour stroma and upregulate immune cell trafficking, which would also assist in shifting tumours to a more immune-inflamed phenotype.

Along with the changes in immune cell populations and their locations within the tumour microenvironment, the cytokine release that occurs with ablation has been shown in most circumstances to create a pro-inflammatory tumour microenvironment, the hope is that these changes can either alter the cancer immune setpoint for these patients, or modify the balance to a more immune stimulatory phase that shifts the patient/tumour over the threshold needed to respond to ICI.

This hypothesis raises the exciting prospect that the potential of ICI therapy could be further enhanced by combination with ablative local therapy. Initial studies in other cancers would suggest that combination treatment can produce a more durable immune response with a reasonable safety profile.

## What is the next step?

At present we still need a more robust assessment of the immune response induced by thermal ablation. Human studies in NSCLC where the tumour microenvironment is assessed pre and post ablation would be particularly powerful. Assessment of immune cell populations and cytokines, their location and function with techniques such as RNA sequencing and mass cytometry and multiplex immunohistochemistry would allow for detailed analysis of changes with ablation (139-141). Investigation of CD8+ and CD4+ T cells would be especially important as the

 

presence and activation status of these cells appear to be an indicator of a strong anti-cancer immune response (43). Once a robust immune response has been demonstrated in NSCLC, performing clinical studies demonstrating tolerability/safety of the combination, and improved treatment outcomes will be needed.

The correlation between response to ablative immunotherapy and other suggested biomarkers predictive of response to ICI such as tumour mutational burden (TMB) would also be important. TMB is the total number of nonsynonymous mutations in the coding regions of genes, and recent evidence suggests that this is a powerful biomarker for selecting patients that will respond to immunotherapy (142). At present PD-L1 expression is used clinically to predict response to ICI, though more recent studies have demonstrated that the response to ICI might be independent of PD-1/PD-L1 expression (33). PD-1 and PD-L1 expression can be altered with ablation, and examining the potential effect of ablation on TMB would be of interest. TMB represent intrinsic tumour properties and alteration in TMB would provide insights into the possible changes to the cancer immune set point. Serial assessment of these biomarkers may be undertaken bronchoscopically to evaluate response to thermal ablation or ablative immunotherapy (143). Understanding responses within the innate intrinsic immune system (144,145) and how these impact responses to immunotherapy will also be important in integrating thermal tumour ablation into treatment paradigms.

## Conclusions

ICI therapy has transformed treatment responses in NSCLC, though responses remain limited to a minority of patients. Immunogenic responses to tumour ablation are well established, and emerging evidence suggests that combination tumour ablation and immunotherapy may augment anti-tumour immune responses. Prospective studies are underway examining clinical efficacy of combination therapy, and studies should focus on both neoadjuvant and metastatic disease settings. Development of delivery systems with improved safety profiles is needed. Bronchoscopic thermal ablative techniques, which are currently under development may achieve this.

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