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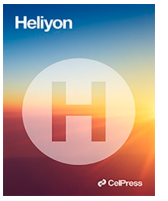

Review article

Local ablative therapies and the effect on antitumor immune responses in pancreatic cancer – A review

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease, projected to rank as the second most prevalent cause of cancer-related mortality by 2030. Despite significant progress in advances in surgical techniques and chemotherapy protocols, the overall survival (OS) remains to be less than 10 % for all stages combined.

In recent years, local ablative techniques have been introduced and utilized as additional therapeutic approaches for locally advanced pancreatic cancer (LAPC), with promising results with respect to local tumor control and OS. In addition to successful cytoreduction, there is emerging evidence that local ablation induces antitumor immune activity that could prevent or even treat distant metastatic tumors. The enhancement of antitumor immune responses could potentially make ablative therapy a therapeutic option for the treatment of metastatic PDAC. In this review, we summarize current ablative techniques used in the management of LAPC and their impact on systemic immune responses.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with an increasing incidence, predicted to become the second leading cause of cancer-related death by 2030 [1]. Despite advances in surgical techniques and more effective systemic chemotherapy regimens, 5-year overall survival (OS) still remains less than 10 % for all stages combined [2]. Although surgical resection is still considered the only chance for cure, fewer than 20 % of patients present with resectable disease [3]. The remaining approximately 30 % of patients are affected by locally advanced pancreatic cancer (LAPC) that is considered unresectable on the basis of arterial involvement (superior mesenteric artery, celiac trunk, or common hepatic artery), or involvement of the mesenteric-portal venous axis that precludes the possibility of venous reconstruction after resection [4].

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With the availability of more effective chemotherapy regimens, neoadjuvant chemotherapy has been more broadly used in recent years for the treatment of selected LAPC. Resection rates for tumors initially classified as unresectable have been reported to be as high as 60 % after neoadjuvant therapy, although many studies have included a mix of unresectable and "borderline resectable" tumors [5–7]. However, even after early systemic treatment, micrometastatic disease is often not controlled as almost 80 % of patients who have undergone "complete" (R0) resection after systemic neoadjuvant therapy will relapse and succumb to their disease due to local recurrence and/or distant metastatic disease [8,9].

In order to improve local tumor control in patients who are not candidates for resection, several local ablative techniques have emerged as additional therapeutic approaches in the multimodal treatment of LAPC [10,11]. Although not explicitly referred to as "ablation", radiation therapy is the most widely utilized ablative therapy for LAPC. Other more direct catheter-based tumor ablation techniques such as radiofrequency ablation (RFA) or microwave ablation (MWA) rely on thermal coagulation to induce cytoreduction, mainly through necrosis. In contrast, irreversible electroporation (IRE) is a non-thermal ablative technique that delivers high-voltage electrical pulses and avoids significant heating of the tissue. This decreases the risk of thermal damage to vital structures adjacent to the ablation zone, such as blood vessels, and cell death is mainly induced through apoptosis [12–14].

Besides local cytoreduction, there is growing evidence that ablative techniques may lead to systemic tumor control through the release of antigens that elicit anti-tumor specific immune responses [15–17]. In theory, cell remnants following ablation remain available for uptake by antigen presenting cells, such as dendritic cells (DCs), which can—in turn—activate T-cells and adaptive immunity [18–20]. It is thought that the increase in immunogenicity will not only kill the primary tumor but also cancer cells distant to the original site of treatment [21]. This phenomenon of spontaneous regression of tumor lesions outside of the field of local treatment – also known as "abscopal effects"—was first described in the 1950s by Mole, referring to an immune-mediated response to radiation [22–24]. The mechanisms behind abscopal effects have not been completely elucidated, but the idea of being able to enhance the anti-tumor immune responses induced by local ablation is obviously appealing. These "abscopal effects" have been studied extensively in preclinical and clinical studies and have been primarily demonstrated in hepatocellular carcinoma, as well as colorectal and other metastatic liver tumors, since these are technically easier to ablate than primary pancreatic tumors [25–28].

In this review, we will provide an overview of local ablative techniques currently in clinical use for LAPC and their ability to induce abscopal effects.

2. Methods

A literature research using PubMed was performed to summarize the most current publications on the use of local ablative therapies in LAPD. Peer-reviewed publications which assessed anti-tumor immune responses as a primary endpoint were included. Based on the data obtained and on the clinical relevance of local ablation currently being used, we focused on the following techniques (Figs. 1 and 2; see Table 1): Radiofrequency Ablation (RFA), Microwave Ablation (MWA), Cryoablation, High-intensity focused ultrasound (HIFU), and Irreversible Electroporation (IRE). Given that the "abscopal effect, identified in the field of radiotherapy, is

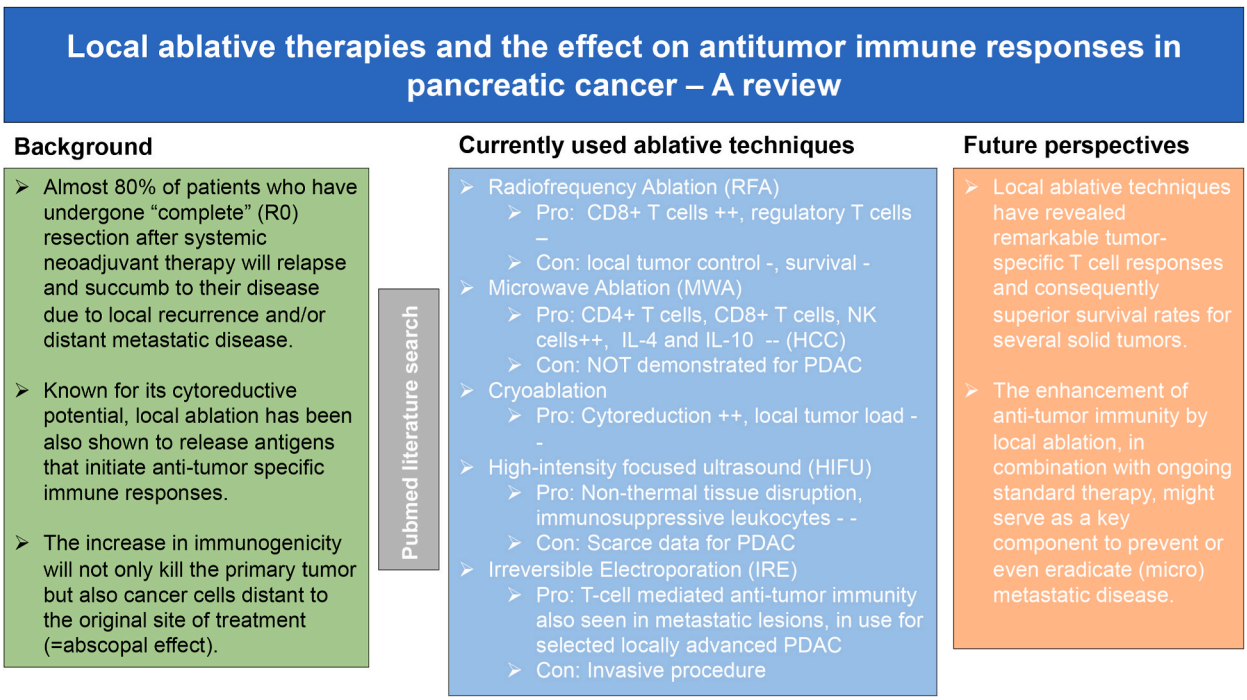

Fig. 1. Local ablative therapies and the effect on antitumor immune responses in PDAC.

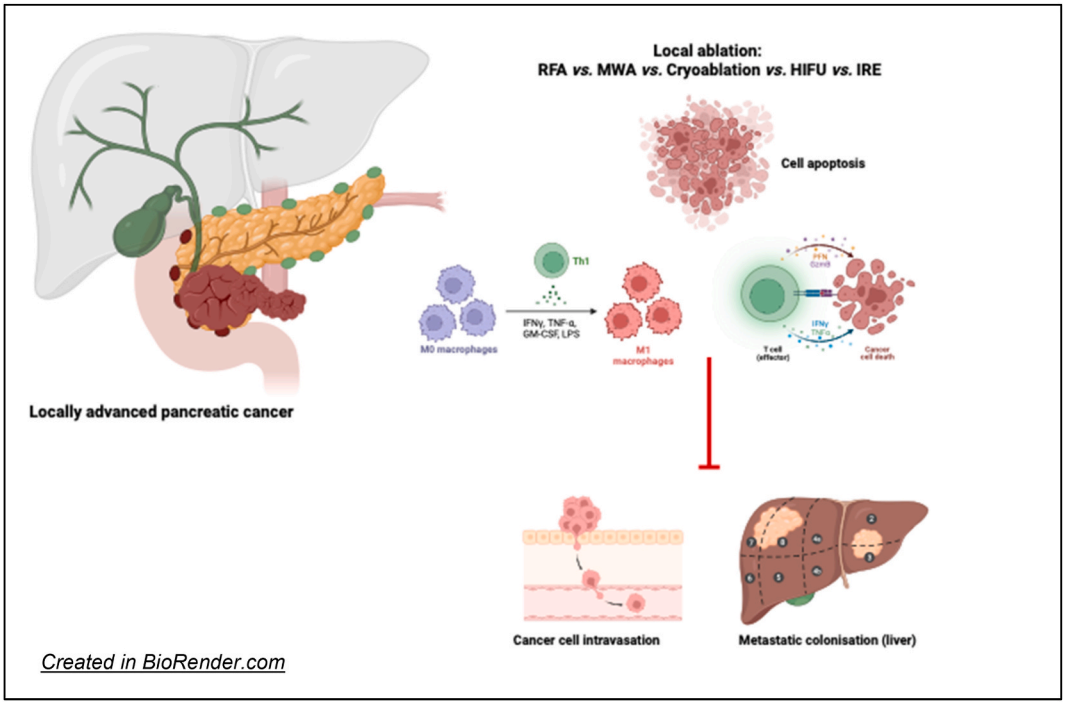

Fig. 2. Antitumor immune responses induced by local ablation.

marked by a decrease in the size of tumors located beyond the radiation zone. Initially observed and documented by Mole, this phenomenon entails a notable reduction in the volume of untreated tumors subsequent to radiotherapy administered for either primary or metastatic tumors.

Given that the "abscopal effect has been identified primarily in the field of radiation therapy (RT), we have also included a brief section of PDAC relevant research related to RT (see also Table 1).

3. Local ablative techniques for pancreatic cancer

3.1. Radiation therapy (RT)

The role of RT in the management of LAPC has been questioned in several clinical trials and remains controversial, although still being incorporated in the NCCN guidelines as a treatment option for borderline resectable and locally advanced tumors [4]. Some studies have suggested that induction chemotherapy before concurrent chemoradiotherapy improves survival [29–32], whereas a randomized controlled trial (LAP-07) failed to demonstrate an overall survival benefit of conventional chemoradiation therapy after induction chemotherapy versus chemotherapy alone [33]. The major disadvantage associated with conventional RT is that PDAC is known to be radioresistant, while the surrounding organs (i.e., stomach, duodenum) are highly radiosensitive and therefore, increase the risk for RT related complications such as bleeding, and perforation [34–37].

The potential of RT to induce anti-tumor specific immune responses that kill the primary tumor and cancer cells distant to the original site of treatment as abscopal effect has been investigated for decades [38,39]. It has been demonstrated that RT can enhance immune responses or the efficacy of immunotherapy by the upregulation of major histocompatibility complex (MHC) class I molecules that are recognized by CD8 T cells [40]. RT has also been associated with the activation of DCs that enhance cross-presentation of tumor antigens and consequently prime an adaptive immune response [41,42].In the last several years, stereotactic body RT (SBRT) has emerged as a potentially more effective RT for LAPC since it allows for precise radiation delivery to the pancreatic tumor with less radiation to the surrounding organs [23,43]. To evaluate the efficacy of SBRT for local control, survival and safety, a retrospective study was performed to analyze patients with LAPC treated with a dose of 45 Gy in 6 fractions [44]. One-, 2- and 3-year local control (LC) rate was 81.9 %, 69.1 % and 58.5 %, while median DPFS was 6.03 months and median OS was 11.6 months. In addition to this, no patients experienced G3 toxicity.

In order to assess the safety of SBRT, a prospective multicentre phase-1 dose-escalation study was performed in BRPC patients, who received pre-operative SBRT, with one dose to the primary tumour and an integrated boost to the region where tumour was in contact with vasculature [45]. Twelve patients were registered, and eleven received SBRT. Nine serious adverse reactions or events occurred (seven CTCAE Grade 3, two Grade 4). Median overall survival for SBRT patients was 8.1 months.

Another study has reported a greater than 10 % risk of gastrointestinal ulceration, which is higher than usually associated with

Table 1
Summary of relevant preclinical and clinical studies assessing therapeutic efficacy and immunomodulatory effects of local ablative therapies for PDAC.

RT Author	Type of study	Tumor stage	Treatment	Primary endpoint	Results	Conclusion
Zhu et al. rowhead	Phase 2 trial	Postop. local recurrence	1. SBRT + pembrolizumab + trametinib (SBRT + K + M) *vs* 2. SBRT + gemcitabine (SBRT + G)	OS, PFS	Longer OS in SBRT (≥65Gy) + K + M (median: 15.1 vs. 12.4 months, HR 0.67 [95%CI 0.43–1.04]; p = 0.071) Longer PFS in SBRT (≥65Gy) + K + M (median: 8.6 vs. 5.0 months, HR 0.48 [95 % CI 0.31–0.77]; p = 0.0021)	Dose escalation of SBRT may improve PFS with pembrolizumab and trametnib. No statistical relevance for longer OS.
Petrelli et al. rowhead	Systematic rev.	Stage III (not resectable)	SBRT	1-year OS	Pooled 1-year OS = 51.6 % in 13 trials. Median OS ranged from 5.7 to 47 months (median 17).	SBRT shows satisfactory OS for inoperable PDAC.
Yasmin-Karim et al. rowhead	Preclinical research	Mouse SQ model (Panc02)	SBRT + intratumoral agonistic anti-CD40	Unilateral treatment of tumor: Abscopal effect?	Single dose SBRT + CD40 lead to regression of contralateral, untreated tumor and result in prolonged survival (p < 0.0001) T cell infiltration increased in combination therapy (p < 0.0001)	SBRT and anti-CD40 effective at augmenting T cell priming, tresulting in vitiligo in long-term survivors.
Azad et al. rowhead	Preclinical research	Mouse SQ model (KPC, Pan02)	1. RT + gemcitabine + anti PD-L1 *Vs* 2. RT + gemcitabine	Does PD-L1 inhibition alter radio- and chemosensitivity?	anti-PD-L1 t + high RT doses (12, 5 × 3, 20 Gy) improved tumor response in KPC and Pan02 allografts. anti PD-L1 + high RT doses (12 Gy) prevent liver metastases	Synergy between high dose RT and anti PD-L1.
Comito et al. rowhead	Retrospective study	Stage III (LAPC)	SBRT (45 Gy in 6 fractions)	Local control (LC), distant	1-, 2- and 3-year LC rate = 81.9 %,	SBRT increased LC. SBRT + chemotherapy improved OS.

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Table 1 (*continued*)

				progression free survival (DPFS), (OS), toxicity	69.1 % and 58.5 %. 1- and 2-year DPFS rate = 19.9 % and 4.5 %. 1-, 2- and 3-year OS rates = 45.4 %, 16.1 %, and 9.8 %. No patients experienced G3 toxicity.	
RFA						
Author	Type of study	Tumor stage	Treatment	Primary endpoint	Results	Conclusion
Fegrachi et al.	Phase II trial	Stage III (LAPC)	RFA	Safety (major complications Clavien-Dindo grade $\geq$ III)	Delayed gastric emptying (DGE) in 4 patients (24 %), 5 patients (29 %) had a major complication other than DGE. 1 (6 %) RFA-related major complications occurred.	RFA is a safe procedure for patients with LAPC.
Gao et al.	Preclinical (Panc02) and basic (human pancreatic cell line) research	Stage III	RFA	Quantifification of immune cell subtypes and related cytokines to identify combination therapies.	Tumor-infiltrating $CD8^+$ T cells increased; regulatory T cells (Tregs) decreased post-RFA treatment. RFA + mTOR inhibitor = synergetic repressive effect on tumor growth	RFA + mTOR signaling blockade can promote tumor immune response, but also restrain residual cancer cell proliferation.
Giardino et al.	Prospective clinical study	Stage III (LAPC)	RFA	Immune reaction/cell infiltration assessed in peripheral Blood samples preoperatively and on post-operative days 3–30.	$CD4^+$, $CD8^+$ and T effector memory cells (TEM) increased from day 3 (=activation of the adaptive response). Myeloid DCs (=tumour-associated antigens) increased at day 30.	This study provides the first evidence of RFA-based immunomodulation in LAPC.

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Table 1 (*continued*)

Faraoni et al.	Preclinical study	Stage III (LAPC)	RFA	Local and abscopal affects after RFA.	RFA reduced PDAC tumor progression RFA elevated dendritic cell numbers in RFA-treated tumors and promoted a significant $CD4^+$ and $CD8^+$ T-cell abscopal response. RFA elevated levels of PD-L1; checkpoint blockade inhibition sustained tumor growth reduction in the context of RFA	RFA promotes antitumor immunity.
Frigerio et al.	Multicenter RCT	LAPC	RFA (Group A) vs. conventional chemoradiotherapy (CHRT, Group B))	OS, PFS	No statistically significant survival benefit from RFA compared to CHRT, neither in terms of OS (medians of 14.2 months and 18.1 months, respectively, $p = 0.639$) nor PFS (medians of 8 months and 6 months respectively, $p = 0.570$).	Compared to CHRT, RFA alone did not provide any advantage in terms of OS or PFS.
MWA						
Author	Type of study	Tumor stage	Treatment	Primary endpoint	Outcome	Conclusion
Ierardi et al. rowhead	Retrospective study	Stage III (LAPC)	MWA	Feasibility and safety	Procedure was feasible in all patients (100 %). Mean hospital stay = 4 days. No major complications.	Percutaneous MWA is a feasible and safe approach for the palliative treatment of advanced stage PDAC.
Vogl et al. rowhead	Retrospective study	Stage III (LAPC)	MWA	Safety, efficacy	No major complications. 2 patients	MWA for LAPC shows promising results regarding feasibility and safety.

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Table 1 (*continued*)

					(9.1 %) with minor complications (severe local pain related to MWA). Local tumor progression in 1 case (10 %) of the 10/22 available three-month follow-up imaging studies.	
Carrafiello et al. rowhead	Retrospective study	Stage III (LAPC)	MWA	Safety and efficacy	Feasible in 100 %. 1 late major complication, no visceral injury was detected.	MWA ablation is a feasible approach in the palliative treatment of pancreatic tumors.
Cryoablation						
Author	Type of study	PDAC stage	Treatment	Primary endpoint	Results	Conclusion
Li et al. rowhead	Retrospective study	Stage IV	Palliative bypass with cryoablation (PBC) vs without	Safety and efficacy	No significant difference in overall complications ($p = 0.809$). Higher delayed gastric emptying rate in the PBC group (36.8 % vs 16.2 %, $p = 0.005$).	Cryosurgery combined with palliative bypass surgery can be considered a safe and effective treatment for unresectable pancreatic cancer.
Song et al. rowhead	Retrospective study	Stage IV	Palliative bypass surgery combined with cryoablation vs. without cryoablation	Safety and efficacy	Reduction in tumor size and CA 19-9 level only in the combination treatment group. No difference in postoperative complications and prognosis.	Cryoablation can reduce tumor size and relieve the patients' symptoms, without improving the patients' prognosis significantly.
HIFU						

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Table 1 (*continued*)

Author	Type of study	Tumor stage	Treatment	Primary endpoint	Results	Conclusion
Sung et al. rowhead	Prospective study	Stage III/IV	HIFU	Safety and efficacy	OS rates at 6, 12, and 18 months from HIFU were 52.2 %, 30.4 %, and 21.79 %, respectively; median survival = 7.0 months.	High-intensity focused ultrasound is safe and effective.
Lee et al. rowhead	Prospective study	Stage III (LAPC)	Concurrent chemotherapy + HIFU (CCHT) vs. Chemotherapy alone	OS, time to tumor progression (TTP), the complications and the current performance status.	OS in the CCHT group (3 patients) = 26.0, 21.6 and 10.8 months. TTP of the three patients in the CCHT group was 13.4, 11.5 and 9.9 months.	This study shows that CCHT is a potentially effective and safe modality for the treatment of unresectable pancreatic cancer.
Zhu et al. rowhead	Retrosepctive study	Stage III (LAPC)	HIFU	Efficacy, pain relief, and relative complications of HIFU therapy. Overall survival rate (OSR) and median survival time (MST).	Pain reduction in 74/86 (86.05 %) patients, total remission rate = 97.6 % (74/76). Total MST = 9.9 months (2–58.7 months), total OSR in 1 and 2 years = 41.5 % and 9.6 %, respectively.	HIFU can significantly alleviate cancer-related pain and prolong the survival time of patients with pancreatic cancer.
Yu et al. rowhead	Preclinical study	Stage III (PDAC xenograft model)	HIFU + microbubbles vs. HIFU + microbubbles + gemcitabine (HIGEM + MB)	Therapeutic effects of combination therapy.	HIGEM + MB group: Higher apoptosis rates ($p < 0.05$), slowest tumor growth.	HIFU combined with microbubbles enhances the therapeutic effects of gemcitabine chemotherapy in a pancreatic cancer xenograft model.
Li et al. rowhead	Retrospective analysis	Stage IV (gemcitabine refractory)	Group A: HIFU + S-1 vs Group B: S-1 alone	OS, PFS	Median OS longer in group A (10.3 months vs. 6.6 months, P = 0.000). Median PFS longer in group A t (5.1 months vs 2.3 months, P = 0.000).	HIFU in combination with S-1 might be effective and well tolerated as salvage chemotherapy in the treatment for metastatic pancreatic cancer.
Wang et al. rowhead	Prospective study	Stage IV	HIFU	Efficacy	NK cell enhanced in 10 patients ($P < 0.05$). \CD3+ and $CD4^+$ subsets, CD4+/	HIFU may enhance cell-mediated immunity in the host.

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Table 1 (*continued*)

					CD8+ ratios increased.	
Zhou et al. rowhead	System. Rev., Meta-Analysis	Stage IV	HIFU alone and/or in combination with chemo/and radiotherapy	Eifficacy	Increased NK cell activity, the population of $CD4^+$ lymphocytes, and the ratio of CD4+/CD8+ in the blood circulation of cancer patients are found after HIFU.	Immune response induced by HIFU ablation may become an effective way of cancer treatment.
IRE						
Author	Type of study	Tumor stage	Treatment	Primary endpoint	Results	Conclusion
Martin et al. rowhead	Prospective study	LAPC	IRE	Safety, efficacy	All patients underwent successful IRE. 1 90-day mortality. No evidence of clinical pancreatitis or fistula formation.	IRE ablation of locally advanced pancreatic cancer tumors is a safe and feasible primary local treatment in unresectable, locally advanced disease.
Martin et al. rowhead	Prospective multi-center study	LAPC	IRE	OS	In a comparison of IRE patients to standard therapy, improved local progression-free survival (14 vs. 6 months, $p = 0.01$), distant progression-free survival (15 vs. 9 months, $p = 0.02$), and overall survival (20 vs. 13 months, $p = 0.03$).	IRE ablation of locally advanced pancreatic tumors remains safe and in the appropriate patient who has undergone standard induction therapy for a minimum of 4 months can achieve greater local palliation and potential improved overall survival compared with standard chemoradiation-chemotherapy treatments.
He et al. rowhead	Preclinical study	LAPC	IRE	Immunomodulatory effects	Tumor growth significantly suppressed, increased infiltration of $CD8^+$ T cells. The growth of untreated tumors was suppressed and the effector $CD8^+$ T cells and memory T cells increased significantly in mice.	IRE induced local immunomodulation by increasing specific T cells infiltration.

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Table 1 (*continued*)

He et al. rowhead	Prospective study	LAPC	IRE	Immunomodulatory effects	The alteration of $CD8^+$ T cell between day 3 and 7 was identified as a prognostic factor for OS and PFS. ROC curve (AUC) and C-indexes of the alteration of $CD8^+$ T cell for OS and PFS = 0.816 and 0.773 and 0.816 and 0.639, respectively.	This study presented the first evidence of IRE-based immunomodulatory in patients with LAPC. The alteration of $CD8^+$ T cell between D3 and D7 showed relatively good performance and could be used as an effective tool for prognostic evaluation for LAPC patients after IRE.
Shankara et al. rowhead	Preclinical study	LAPC	IRE + CD40 agonistic antibody	Therapeutic immune effects	IRE + CD40 Ab improved median survival >35 days, (vs. 21 days for IRE and 24 days CD40Ab, $p < 0.01$). CD40Ab decreased metastatic disease burden, with less disease in the combination group than in the sham group or IRE alone.	Addition of CD40Ab to IRE improved dendritic cell activation and neoantigen recognition, while generating a strong systemic antitumor T-cell response that inhibited metastatic disease progression.

conventional RT [46].

Notably, there is evidence that the combination of RT with immunotherapy can improve local and distant tumor control as a result of improved anti-tumor immune responses in several preclinical studies [47–50]. In a preclinical mouse model of PDAC, the combination of a single dose of SBRT with intratumoral injection of agonistic anti-CD40, resulted in regression of non-treated contralateral tumors and formation of long-term immunologic memory [51]. Another study group has demonstrated that the addition of anti-PD-L1 to high doses of radiation therapy has significantly improved tumor response in preclinical PDAC models [52]. In addition to this, they showed that PD-L1 blockade further augmented the effect of high RT doses (12 Gy) in preventing development of liver metastases.

These results have provided preclinical for the approach of combining RT with immuno therapy for several tumor entities, including PDAC, and several early-stage clinical studies are ongoing.

3.2. Radiofrequency ablation (RFA)

Radiofrequency ablation (RFA) is a thermal ablative method that generates hyperthermic temperatures through high-frequency alternating current that induces coagulation and protein denaturation and eventually results in tumor destruction through necrosis [53–55]. RFA is in clinical use primarily for hepatocellular carcinoma (HCC) and colorectal liver metastases [56]. A commonly described limitation of RFA is the “heat-sink effect” that decreases susceptibility of the tumor to thermal damage when adjacent to larger vessels, as occurs frequently in LAPC [16,53]. Consequently, the use of RFA in PDAC is limited, although newer studies also outline its safety and feasibility in the treatment of LAPC [57,58]. Moreover, some research groups have illustrated

that RFA can impact tumor lesions beyond the region subjected to ablation, suggesting its potential as an immunomodulatory therapy [59–61]. Nevertheless, experiments in preclinical settings utilizing the Panc02 cell line in a murine pancreatic tumor model indicated no significant impact on local tumor growth after RFA treatment [59]. However, there was a notable increase in the proportion of $CD8^+$ T cells infiltrating the tumor, accompanied by a decrease in the number of regulatory T cells (Tregs) following RFA treatment. Subsequently, combining RFA treatment with an mTOR inhibitor demonstrated a synergistic inhibitory effect on tumor growth in this preclinical murine cancer model [59].

In a prospective clinical study, peripheral blood samples of patients with LAPC after RFA were obtained preoperatively and on postoperative days 3–30 [59]. The results revealed a general activation of adaptive immune responses by an increase of $CD4^+$ and $CD8^+$ T-cells. In addition to this, they revealed a remarkable increase in effector memory T cells (TEMs) which is known to be an important step in a systemic immune response. Taken together, the investigators concluded that RFA induces immunomodulation in LAPC. Nonetheless, despite some promising preclinical studies and a few clinical studies demonstrating safety, the first randomized controlled trial comparing open surgical RFA to conventional chemoradiation in 100 patients with LAPC compellingly showed no benefit for RFA [60]. The increased availability of endoscopic ultrasound (EUS)-guided RFA has renewed interest in this modality, but a phase II randomized controlled trial comparing EUS-guided RFA plus chemotherapy to chemotherapy alone in borderline resectable or LAPC also failed to demonstrate a survival benefit [61].

Due to the absence of randomized controlled trials (RCTs) exploring the impact of combining radiofrequency ablation (RFA) with established chemotherapy on overall survival (OS), the "Pancreatic Locally Advanced Unresectable Cancer Ablation" (PELICAN) trial has been launched [62]. This multicenter superiority RCT enrolls all LAPC patients initiating FOLFIRINOX or (nab-paclitaxel/)gemcitabine, subject to eligibility screening. Following restaging, patients with stable disease or objective response according to RECIST criteria, for whom resection is not viable, undergo randomization to either RFA followed by chemotherapy or chemotherapy alone. A total of 228 patients from 16 centers in The Netherlands and four other European centers are included, with the primary endpoint being overall survival.

3.3. Microwave ablation (MWA)

Unlike RFA, the coagulative effect of MWA is generated by water molecule oscillations caused by an alternating electromagnetic field that creates dielectric instead of frictional heating [63–65]. Although a major advantage of MWA, compared to RFA, is its effectiveness in different tissue types (e.g., fibrous and charred tissues), the extent of the ablation zone is less predictable and explains the controversy concerning the possible side effects related to this technique [53]. Therefore, the literature for MWA for LAPC is even more scarce than for RFA, and results from existing studies are not encouraging given that progressive disease following MWA in LAPC was detected in the majority of patients [64,66].

The immunomodulatory effects of MWA have primarily been assessed for hepatocellular carcinoma (HCC) showing an increase of immune stimulatory cells (i.e., $CD4^+$ T cells, $CD8^+$ T cells, NK cells) and a decrease in immunosuppressive cells (i.e., IL-4 and IL-10), associated with better survival and lower recurrence rates [26,67,68]. As such, in a prospective clinical study, peripheral blood samples from HCC patients showed a significant increase in $CD3^+$, $CD4^+$ cells and IL-12 one month after MWA which was associated with an improved anti-tumor immunity [67]. This was confirmed by another study group showing an increase in immune infiltrating cells in tumor tissues 3 and 17 days after MWA. The immune modulatory effects of MWA have not been demonstrated for PDAC yet and need to be confirmed in future studies.

3.4. Cryoablation

Cryoablation causes coagulative necrosis and cell death by using liquefied gases that cool as they expand, such as argon nitrogen and helium gases [69]. Two retrospective studies have assessed the safety and efficacy of cryoablation in the treatment for unresectable

PDAC [70,71]. In both studies, cytoreduction after treatment was successful and local tumor load was reduced. However, cryoablation did not improve OS [70,71]. Evidence for immune effects of cryoablation in PDAC is limited. One of few studies directly comparing the immune effects of different ablation techniques (using the B16 melanoma cell line) showed that cryoablation produced a greater release of total and native (non-denatured) proteins than ablation with heat [72]. They also demonstrated that cryoablation was associated with an enhanced release of a melanoma antigen (TRP-2) and antigen-specific T-cell activation than heat, although non-thermal ablation with irreversible electroporation (IRE) produced a significantly higher antigen release and T-cell activation than either thermal technique [72].

In a recent publication, researchers investigated the abscopal effect following cryoablation on bone metastasis using a mouse model [73].

In this study, a breast cancer cell line was implanted into the bilateral tibiae of the animals, with one tumor (left) undergoing local cryoablation treatment. The results indicated an impact of cryoablation alone on the volume of the untreated tumor on the opposite side (right). Furthermore, the combination of cryoablation with anti-PD-1 exhibited a significant immunoenhancing effect [75].

Although the investigators suggest that cryoablation confirms an abscopal effect which can be enhanced by combination therapy with immune anti-PD-1, reliable data for PDAC remain scarce.

3.5. *High-intensity focused ultrasound (HIFU)*

HIFU is a newer ablative technique and the only non-invasive modality that can generate hyperthermic temperatures by delivering high-intensity ultrasound beams in a target definite area of interest [74,75]. Subsequently, this selectively treated area is destroyed by coagulative necrosis while the tissue outside of this area remains intact [76]. Several studies have demonstrated the feasibility and safety of HIFU in the treatment of pancreatic tumors, primarily in a palliative setting for unresectable PDAC [77,78]. A few non-randomized studies have suggested beneficial effects of HIFU—either as monotherapy or in combination with chemotherapy—on pain control as well as some objective tumor responses and prolonged median survival [77,79–81]. Immunomodulatory effects following HIFU have been reported by a study group, which has evaluated blood samples of 15 patients with late stage PDAC before and after HIFU therapy [82]. They demonstrated an increase in circulating $CD3^+$ and CD4 T cells, an increased CD4+/CD8+ T-cell ratio, and increased NK cell activity. The immunemodulatory effect of HIFU has been also supported by by a meta-analysis of 3022 clinical PDAC cases that had been treated with HIFU [79].

Another aspect of HIFU is that it can be also used to induce non-thermal effects for disruption of tissue, a technique that is known as histotripsy [83]. This ability to avoid thermal destruction and denaturation of proteins is of particular interest as it is expected to enhance anti-tumor specific immune responses [84]. A recent preclinical study demonstrated that histotripsy was superior to thermal ablation in its ability to release potential antigens from a murine PDAC cell line *in vitro*; treatment of subcutaneous tumors *in vivo* induced a decrease in immunosuppressive tumor-infiltrating leukocytes and prolonged survival [85]. Further investigations are clearly warranted to elaborate the immunomodulatory effects of HIFU and histotripsy ablation.

3.6. *Irreversible electroporation (IRE)*

IRE is a non-thermal local ablation therapy that uses high-voltage (maximum 3000 V), short microsecond pulse lengths (70–90μs) to induce permanent cell membrane damage and cellular apoptosis [10]. Since 2009, IRE has been used clinically in selected patients with LAPC who have not demonstrated distant progression after neoadjuvant chemotherapy, with studies showing median OS similar to patients undergoing resection (25 months) [86,87]. As opposed to thermal ablative techniques, IRE is believed to have the distinct advantage that cell death is caused by disruption of the cellular membrane integrity and therefore results in relative preservation of adjacent surrounding structures (i.e., bile ducts, larger blood vessels) [88–90]. This and the fact that it is not vulnerable to “heat sink” effects render IRE particularly attractive to treat LAPC [88].

Presumably, immune stimulation induced by IRE may be more pronounced compared to thermal ablation since it has been shown that different ablative mechanisms result in varying levels of immune cell infiltration [14,91,92]. A preclinical study by Bulvik et al. evaluated the effects of IRE and RFA in an orthotopic hepatocellular carcinoma (HCC) mouse model and in a subcutaneous HCC xenograft mouse model [89]. In this study, infiltration of leukocytes into the ablation zone was present in IRE-treated, but not in RFA-treated lesions. Furthermore, ablation of the liver with IRE slowed the growth of remote, untreated subcutaneous tumors, which was more pronounced compared to RFA. Indeed, the immunomodulatory and abscopal effects of IRE have been assessed extensively in non-pancreatic cancers and, more recently—in preclinical PDAC models and clinical studies [14,15,91,93].

Using a subcutaneous mouse model, our laboratory showed that surgical resection was more effective at controlling the primary tumor compared to IRE [15]. However, when tumor-free mice were rechallenged with injection of live tumor cells on the contralateral flank, 3 of 5 mice treated with resection showed secondary tumor growth while the animals from the IRE group remained tumor-free. These findings demonstrate that IRE can generate protective immunity. We furthermore demonstrated that protective immunity is T-cell mediated through adoptive transfer experiments. Scheffer et al. assessed the immune modulatory effects of IRE by obtaining peripheral blood samples pre-and post-IRE treatment of patients with LAPC who were enrolled in the PANFIRE clinical trial [12,14]. Flow cytometric analysis revealed a transient decrease in systemic regulatory T cells (Treg) and a simultaneous transient increase in activated PD-1^+ T cells, which recent evidence indicates can identify-tumor specific T cells in the tumor microenvironment and in peripheral blood [94]. Another group from China explored peripheral blood samples from 34 patients with LAPC, collected before surgery and on day three and seven after IRE, respectively [92]. Their findings indicated a change in $CD8^+$ T cells between days three and seven, correlating with enhanced overall survival (OS) and progression-free survival (PFS) following IRE. This aligns with earlier

research suggesting a higher incidence of metastatic lesions in patients with reduced immune cell infiltrates, suggesting that systemic changes could potentially induce abscopal effects [95].

However, although these studies have demonstrated promising results, it has also been shown that the systemic immune effects following IRE alone are generally not sufficient to induce clinical abscopal effects. Established immune-tolerance mechanisms induced by the primary tumor and (micro)metastatic lesions might decrease the development of robust clinical responses [24]. To overcome this limitation and to increase the abscopal effect on distant tumor lesions (i.e., metastasis), many researchers have started to combine IRE with immunotherapy [17,96,97]. A study by Zhao et al. showed that the combination of IRE and anti-programmed cell death protein 1 (anti-PD1) immune checkpoint blockade promotes selective tumor infiltration by $CD8^+$ T cells and significantly prolongs survival in a murine orthotopic PDAC model to a greater extent than did the combination of RT and anti-PD1 therapy [13]. A phase II clinical trial of IRE with adjuvant checkpoint blockade (NCT03080974) is currently ongoing at University of Louisville. They have recently published their results with the first 10 patients, demonstrating that this combination is well-tolerated [98]. Another group's clinical study investigated the impact on OS and PFS of IRE versus IRE combined with NK cell immunotherapy (IRE-NK) in patients with unresectable stage III and IV PC [99]. They showed that median OS (13.2 months vs. 11.4 months) and PFS (9.3 months vs. 8.1 months) was higher in the IRE-NK group compared to IRE alone.

Prior work from our lab has demonstrated that IRE produced complete regression of subcutaneous tumors in up to 30 % of immunocompetent mice. However, the combination of IRE with intratumoral toll-like receptor-7 (TLR7) agonist (1V270) and systemic anti-programmed death-1 receptor (PD)-1 checkpoint blockade resulted in elimination of untreated concomitant distant tumors [15]. The PANFIRE-3 trial (NCT04612530) in Europe is currently evaluating a similar strategy, utilizing percutaneous IRE in combination with nivolumab (anti-PD1) and CpG (a TLR9 agonist) for patients with metastatic PDAC. In a more recent work from our lab, we used an orthotopic PDAC model to study the combination of IRE with a single intratumoral injection of an agonistic CD40 antibody at the time of IRE [93]. The combination not only improved local tumor control, but it also significantly decreased metastatic disease burden in the liver and increased infiltration of $CD8^+$ T-cells and activated dendritic cells within liver metastases. A first-in-human study of IT injection of an agonistic CD40 Ab, ADC-1013 or mitazalimab (Alligator Biosciences) has demonstrated that injection even into solid organs is feasible and safe [100], and a clinical trial combining IRE with local delivery of CD40 Ab in LAPC is currently in development.

4. Conclusions and Future perspectives

The biggest challenge in the therapy of LAPC remains the fact that most patients have (micro)metastatic disease at diagnosis that cannot be detected on imaging studies. Systemic chemotherapy is the cornerstone of the multimodal treatment approach for PDAC; however, it has only provided a moderate survival benefit for affected patients. The key component to eradicating (micro)metastatic disease and overcoming the high rates of PDAC recurrence is believed to be the activation of tumor-specific immune responses in the host. PDAC is an aggressive disease, considered as immunologically "cold" and, so far, resistant to immunotherapy. Local ablative techniques have increased in number and availability over the last several years and have been associated with not only local tumor control but also distinct immunomodulatory effects. Studies investigating the systemic immune effects of currently available ablative techniques have revealed remarkable tumor-specific T cell responses and superior survival rates for several solid tumors. Encouragingly, the combination of IRE and immunotherapy in LAPC has also shown potential for improved anti-tumor immunity through increased cytotoxic T cell responses. Both further preclinical investigations and translation of preclinical findings to clinical trials are necessary to develop this promising treatment paradigm into meaningful survival benefits for patients with PDAC.

5. Availability of data and materials

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Data availability statement

Data presented in this study derive from peer-reviewed publications found during our literature research on PubMed. These data are publicly available on PubMed.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Suna Erdem: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Jayanth Shankara Narayanan:** Writing – review & editing. **Mathias Worni:** Writing – review & editing, Supervision. **Martin Bolli:** Writing – review & editing, Supervision. **Rebekah R. White:** Writing – review & editing, Visualization, Validation, Supervision.

Declaration of competing interest

The authors declare that they have **no known competing financial interests or personal relationships** that could have appeared to influence the work reported in this paper.

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References

[1] R.L. Siegel, et al., Cancer statistics, 2023, CA Cancer J Clin 73 (1) (2023) 17–48.
[2] 1975-2018N.A. Howlader N, M. Krapcho, D. Miller, A. Brest, M. Yu, J. Ruhl, Z. Tatalovich, A. Mariotto, D.R. Lewis, H.S. Chen, E.J. Feuer, K.A. Cronin (Eds.), SEER Cancer Statistics Review, National Cancer Institute, Bethesda, MD, 2020 [cited 2021; Available from: https://seer.cancer.gov/csr/1975_2018/.
[3] J. Kleeff, et al., Pancreatic cancer, Nat Rev Dis Primers 2 (2016), 16022.
[4] M.A. Tempero, et al., Pancreatic adenocarcinoma, version 2.2021, NCCN clinical practice guidelines in oncology, J Natl Compr Canc Netw 19 (4) (2021) 439–457.
[5] J.M. Cloyd, et al., Neoadjuvant therapy for resectable and borderline resectable pancreatic cancer: a meta-analysis of randomized controlled trials, J. Clin. Med. 9 (4) (2020).
[6] T. Hackert, et al., Locally advanced pancreatic cancer: neoadjuvant therapy with folfirinox results in resectability in 60% of the patients, Ann. Surg. 264 (3) (2016) 457–463.
[7] E. Versteijne, et al., Meta-analysis comparing upfront surgery with neoadjuvant treatment in patients with resectable or borderline resectable pancreatic cancer, Br. J. Surg. 105 (8) (2018) 946–958.
[8] J.Y. Jang, et al., Oncological benefits of neoadjuvant chemoradiation with gemcitabine versus upfront surgery in patients with borderline resectable pancreatic cancer: a prospective, randomized, open-label, multicenter phase 2/3 trial, Ann. Surg. 268 (2) (2018) 215–222.
[9] D.P.S. Sohal, et al., Efficacy of perioperative chemotherapy for resectable pancreatic adenocarcinoma: a phase 2 randomized clinical trial, JAMA Oncol. 7 (3) (2021) 421–427.
[10] R.C. Martin 2nd, et al., Treatment of 200 locally advanced (stage III) pancreatic adenocarcinoma patients with irreversible electroporation: safety and efficacy, Ann. Surg. 262 (3) (2015) 486–494. ; discussion 492-4.
[11] A.H. Ruarus, et al., Percutaneous irreversible electroporation in locally advanced and recurrent pancreatic cancer (PANFIRE-2): a multicenter, prospective, single-arm, phase II study, Radiology 294 (1) (2020) 212–220.
[12] H.J. Scheffer, et al., Ablation of locally advanced pancreatic cancer with percutaneous irreversible electroporation: results of the phase I/II PANFIRE study, Radiology 282 (2) (2017) 585–597.
[13] J. Zhao, et al., Irreversible electroporation reverses resistance to immune checkpoint blockade in pancreatic cancer, Nat. Commun. 10 (1) (2019) 899.
[14] H.J. Scheffer, et al., Irreversible electroporation of locally advanced pancreatic cancer transiently alleviates immune suppression and creates a window for antitumor T cell activation, OncoImmunology 8 (11) (2019), 1652532.
[15] J.S.S. Narayanan, et al., Irreversible electroporation combined with checkpoint blockade and TLR7 stimulation induces antitumor immunity in a murine pancreatic cancer model, Cancer Immunol. Res. 7 (10) (2019) 1714–1726.
[16] K.F. Chu, D.E. Dupuy, Thermal ablation of tumours: biological mechanisms and advances in therapy, Nat. Rev. Cancer 14 (3) (2014) 199–208.
[17] M. Lin, et al., Irreversible electroporation plus allogenic Vgamma9Vdelta2 T cells enhances antitumor effect for locally advanced pancreatic cancer patients, Signal Transduct Target Ther 5 (1) (2020) 215.
[18] R.M. Brock, et al., Starting a fire without flame: the induction of cell death and inflammation in electroporation-based tumor ablation strategies, Front. Oncol. 10 (2020) 1235.
[19] R.J. van den Bijgaart, et al., Thermal and mechanical high-intensity focused ultrasound: perspectives on tumor ablation, immune effects and combination strategies, Cancer Immunol. Immunother. 66 (2) (2017) 247–258.
[20] A. Makkouk, G.J. Weiner, Cancer immunotherapy and breaking immune tolerance: new approaches to an old challenge, Cancer Res. 75 (1) (2015) 5–10.
[21] T.F. Justesen, et al., Electroporation and immunotherapy-unleashing the abscopal effect, Cancers 14 (12) (2022).
[22] R.H. Mole, Whole body irradiation; radiobiology or medicine? Br. J. Radiol. 26 (305) (1953) 234–241.
[23] A.B. Sharabi, et al., Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy, Lancet Oncol. 16 (13) (2015) e498–e509.
[24] W. Ngwa, et al., Using immunotherapy to boost the abscopal effect, Nat. Rev. Cancer 18 (5) (2018) 313–322.
[25] M.W. Loffler, et al., A non-interventional clinical trial assessing immune responses after radiofrequency ablation of liver metastases from colorectal cancer, Front. Immunol. 10 (2019) 2526.
[26] K. Leuchte, et al., Microwave Ablation Enhances Tumor-specific Immune Response in Patients with Hepatocellular Carcinoma, Cancer Immunol Immunother, 2020.
[27] A.G. Duffy, et al., Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma, J. Hepatol. 66 (3) (2017) 545–551.
[28] J.M. Llovet, et al., Locoregional therapies in the era of molecular and immune treatments for hepatocellular carcinoma, Nat. Rev. Gastroenterol. Hepatol. 18 (5) (2021) 293–313.
[29] P.J. Loehrer, Sr ., et al., Gemcitabine alone versus gemcitabine plus radiotherapy in patients with locally advanced pancreatic cancer: an Eastern Cooperative Oncology Group trial, J. Clin. Oncol. 29 (31) (2011) 4105–4112.
[30] W.F. Regine, et al., Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: a randomized controlled trial, JAMA 299 (9) (2008) 1019–1026.

[31] J.E. Murphy, et al., Total neoadjuvant therapy with FOLFIRINOX followed by individualized chemoradiotherapy for borderline resectable pancreatic adenocarcinoma: a phase 2 clinical trial, JAMA Oncol. 4 (7) (2018) 963–969.
[32] T. Hayashi, et al., Phase 2 study of neoadjuvant treatment of sequential S-1-Based concurrent chemoradiation therapy followed by systemic chemotherapy with gemcitabine for borderline resectable pancreatic adenocarcinoma (HOPS-br 01), Int. J. Radiat. Oncol. Biol. Phys. 105 (3) (2019) 606–617.
[33] P. Hammel, et al., Effect of chemoradiotherapy vs chemotherapy on survival in patients with locally advanced pancreatic cancer controlled after 4 Months of gemcitabine with or without erlotinib: the LAP07 randomized clinical trial, JAMA 315 (17) (2016) 1844–1853.
[34] B. Chauffert, et al., Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study, Ann. Oncol. 19 (9) (2008) 1592–1599.
[35] F. Wang, et al., SMAD4 gene mutation renders pancreatic cancer resistance to radiotherapy through promotion of autophagy, Clin. Cancer Res. 24 (13) (2018) 3176–3185.
[36] Y. Zhang, C.W. Houchen, M. Li, Attenuating DNA damage response and immunosuppression radiosensitizes pancreatic cancer, EBioMedicine 76 (2022), 103822.
[37] J.J. Souchek, et al., Unbiased analysis of pancreatic cancer radiation resistance reveals cholesterol biosynthesis as a novel target for radiosensitisation, Br. J. Cancer 111 (6) (2014) 1139–1149.
[38] S. Demaria, E.B. Golden, S.C. Formenti, Role of local radiation therapy in cancer immunotherapy, JAMA Oncol. 1 (9) (2015) 1325–1332.
[39] S. Demaria, et al., The optimal partnership of radiation and immunotherapy: from preclinical studies to clinical translation, Radiat. Res. 182 (2) (2014) 170–181.
[40] J. Neefjes, et al., Towards a systems understanding of MHC class I and MHC class II antigen presentation, Nat. Rev. Immunol. 11 (12) (2011) 823–836.
[41] A.A. Lugade, et al., Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor, J. Immunol. 174 (12) (2005) 7516–7523.
[42] A. Gupta, et al., Radiotherapy promotes tumor-specific effector CD8+ T cells via dendritic cell activation, J. Immunol. 189 (2) (2012) 558–566.
[43] X. Zhu, et al., Effect of stereotactic body radiotherapy dose escalation plus pembrolizumab and trametinib versus stereotactic body radiotherapy dose escalation plus gemcitabine for locally recurrent pancreatic cancer after surgical resection on survival outcomes: a secondary analysis of an open-label, randomised, controlled, phase 2 trial, EClinicalMedicine 55 (2023), 101764.
[44] T. Comito, et al., Can STEreotactic body radiation therapy (SBRT) improve the prognosis of unresectable locally advanced pancreatic cancer? Long-term clinical outcomes, toxicity and prognostic factors on 142 patients (STEP study), Curr. Oncol. 30 (7) (2023) 7073–7088.
[45] D.L.P. Holyoake, et al., SPARC, a phase-I trial of pre-operative, margin intensified, stereotactic body radiation therapy for pancreatic cancer, Radiother. Oncol. 155 (2021) 278–284.
[46] F. Petrelli, et al., Stereotactic body radiation therapy for locally advanced pancreatic cancer: a systematic review and pooled analysis of 19 trials, Int. J. Radiat. Oncol. Biol. Phys. 97 (2) (2017) 313–322.
[47] L. Deng, et al., Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice, J. Clin. Invest. 124 (2) (2014) 687–695.
[48] S.J. Dovedi, et al., Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade, Cancer Res. 74 (19) (2014) 5458–5468.
[49] S.C. Formenti, S. Demaria, Combining radiotherapy and cancer immunotherapy: a paradigm shift, J Natl Cancer Inst 105 (4) (2013) 256–265.
[50] E.B. Golden, et al., An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer, Cancer Immunol. Res. 1 (6) (2013) 365–372.
[51] S. Yasmin-Karim, et al., Radiation and local anti-CD40 generate an effective in situ vaccine in preclinical models of pancreatic cancer, Front. Immunol. 9 (2018) 2030.
[52] A. Azad, et al., PD-L1 blockade enhances response of pancreatic ductal adenocarcinoma to radiotherapy, EMBO Mol. Med. 9 (2) (2017) 167–180.
[53] B. Geboers, et al., Needle-guided ablation of locally advanced pancreatic cancer: cytoreduction or immunomodulation by in vivo vaccination? Chin. Clin. Oncol. 8 (6) (2019) 61.
[54] M. Ahmed, et al., Principles of and advances in percutaneous ablation, Radiology 258 (2) (2011) 351–369.
[55] S.G.G. Testoni, et al., Systematic review of endoscopy ultrasound-guided thermal ablation treatment for pancreatic cancer, Endosc Ultrasound 9 (2) (2020) 83–100.
[56] NCCN, Clinical Practice Guidelines in Oncology: Hepatobiliary Cancers, 2020. *Version 2020*.
[57] F. Scopelliti, et al., Technique, safety, and feasibility of EUS-guided radiofrequency ablation in unresectable pancreatic cancer, Surg. Endosc. 32 (9) (2018) 4022–4028.
[58] S. Fegrachi, et al., Safety of radiofrequency ablation in patients with locally advanced, unresectable pancreatic cancer: a phase II study, Eur. J. Surg. Oncol. 45 (11) (2019) 2166–2172.
[59] A. Giardino, et al., Immunomodulation after radiofrequency ablation of locally advanced pancreatic cancer by monitoring the immune response in 10 patients, Pancreatology 17 (6) (2017) 962–966.
[60] I. Frigerio, et al., Open radiofrequency ablation as upfront treatment for locally advanced pancreatic cancer: requiem from a randomized controlled trial, Pancreatology 21 (7) (2021) 1342–1348.
[61] S.G.G. Testoni, et al., Efficacy of endoscopic ultrasound-guided ablation with the HybridTherm probe in locally advanced or borderline resectable pancreatic cancer: a phase II randomized controlled trial, Cancers 13 (18) (2021).
[62] M.S. Walma, et al., Radiofrequency ablation and chemotherapy versus chemotherapy alone for locally advanced pancreatic cancer (PELICAN): study protocol for a randomized controlled trial, Trials 22 (1) (2021) 313.
[63] G. Carrafiello, et al., Microwave tumors ablation: principles, clinical applications and review of preliminary experiences, Int. J. Surg. 6 (Suppl 1) (2008) S65–S69.
[64] A.M. Ierardi, et al., Percutaneous microwave thermosphere ablation of pancreatic tumours, Gland Surg. 7 (2) (2018) 59–66.
[65] T.J. Vogl, et al., Microwave ablation of pancreatic tumors, Minim Invasive Ther. Allied Technol. 27 (1) (2018) 33–40.
[66] G. Carrafiello, et al., Microwave ablation of pancreatic head cancer: safety and efficacy, J Vasc Interv Radiol 24 (10) (2013) 1513–1520.
[67] H. Zhang, et al., Effects of microwave ablation on T-cell subsets and cytokines of patients with hepatocellular carcinoma, Minim Invasive Ther. Allied Technol. 26 (4) (2017) 207–211.
[68] X. Duan, et al., Combined use of microwave ablation and cell immunotherapy induces nonspecific immunity of hepatocellular carcinoma model mice, Cell Cycle 19 (24) (2020) 3595–3607.
[69] R.L. Cazzato, et al., Percutaneous image-guided cryoablation: current applications and results in the oncologic field, Med. Oncol. 33 (12) (2016) 140.
[70] J. Li, et al., Tumour cryoablation combined with palliative bypass surgery in the treatment of unresectable pancreatic cancer: a retrospective study of 142 patients, Postgrad Med J 87 (1024) (2011) 89–95.
[71] Z.G. Song, et al., The outcome of cryoablation in treating advanced pancreatic cancer: a comparison with palliative bypass surgery alone, J Dig Dis 15 (10) (2014) 561–569.
[72] Q. Shao, et al., Engineering T cell response to cancer antigens by choice of focal therapeutic conditions, Int. J. Hyperther. 36 (1) (2019) 130–138.
[73] R. Annen, et al., Tumor-specific immunoenhancing effects after local cryoablation for metastatic bone tumor in a mouse model, Int. J. Mol. Sci. 23 (16) (2022).
[74] H.Y. Sung, et al., Long-term outcome of high-intensity focused ultrasound in advanced pancreatic cancer, Pancreas 40 (7) (2011) 1080–1086.
[75] J.Y. Lee, et al., Concurrent chemotherapy and pulsed high-intensity focused ultrasound therapy for the treatment of unresectable pancreatic cancer: initial experiences, Korean J. Radiol. 12 (2) (2011) 176–186.
[76] M. Diana, et al., High intensity focused ultrasound (HIFU) applied to hepato-bilio-pancreatic and the digestive system-current state of the art and future perspectives, Hepatobiliary Surg. Nutr. 5 (4) (2016) 329–344.

[77] M. Marinova, T. Wilhelm-Buchstab, H. Strunk, Advanced pancreatic cancer: high-intensity focused ultrasound (HIFU) and other local ablative therapies, Röfo 191 (3) (2019) 216–227.
[78] B. Zhu, et al., High-intensity focused ultrasound ablation for advanced pancreatic cancer, J. Cancer Res. Therapeut. 15 (4) (2019) 831–835.
[79] Y. Zhou, High-intensity Focused Ultrasound Treatment for Advanced Pancreatic Cancer, vol. 2014, Gastroenterol Res Pract, 2014, 205325.
[80] M.H. Yu, et al., Therapeutic effects of microbubbles added to combined high-intensity focused ultrasound and chemotherapy in a pancreatic cancer xenograft model, Korean J. Radiol. 17 (5) (2016) 779–788.
[81] X. Li, et al., Retrospective analysis of high intensity focused ultrasound combined with S-1 in the treatment of metastatic pancreatic cancer after failure of gemcitabine, Am. J. Cancer Res. 6 (1) (2016) 84–90.
[82] X. Wang, J. Sun, High-intensity focused ultrasound in patients with late-stage pancreatic carcinoma, Chin Med J (Engl) 115 (9) (2002) 1332–1335.
[83] V.A. Khokhlova, et al., Histotripsy methods in mechanical disintegration of tissue: towards clinical applications, Int. J. Hyperther. 31 (2) (2015) 145–162.
[84] F. Wu, L. Zhou, W.R. Chen, Host antitumour immune responses to HIFU ablation, Int. J. Hyperther. 23 (2) (2007) 165–171.
[85] A. Hendricks-Wenger, et al., Histotripsy ablation alters the tumor microenvironment and promotes immune system activation in a subcutaneous model of pancreatic cancer, IEEE Trans Ultrason Ferroelectr Freq Control 68 (9) (2021) 2987–3000.
[86] R.C. Martin 2nd, et al., Irreversible electroporation therapy in the management of locally advanced pancreatic adenocarcinoma, J. Am. Coll. Surg. 215 (3) (2012) 361–369.
[87] R.C. Martin 2nd, et al., Irreversible electroporation in locally advanced pancreatic cancer: potential improved overall survival, Ann. Surg Oncol. 20 (Suppl 3) (2013) S443–S449.
[88] E.W. Lee, C.T. Loh, S.T. Kee, Imaging guided percutaneous irreversible electroporation: ultrasound and immunohistological correlation, Technol. Cancer Res. Treat. 6 (4) (2007) 287–294.
[89] B.E. Bulvik, et al., Irreversible electroporation versus radiofrequency ablation: a comparison of local and systemic effects in a small-animal model, Radiology 280 (2) (2016) 413–424.
[90] F.E.F. Timmer, et al., Irreversible electroporation for locally advanced pancreatic cancer, Tech Vasc Interv Radiol 23 (2) (2020), 100675.
[91] C He, X Huang, Y Zhang, X Lin, S Li, T-cell activation and immune memory enhancement induced by irreversible electroporation in pancreatic cancer, Clin Transl Med 10 (2) (2020 Jun) e39, https://doi.org/10.1002/ctm2.39. Epub 2020 Jun 4. PMID: 32508058; PMCID: PMC7403705.
[92] C. He, et al., Immunomodulatory effect after irreversible electroporation in patients with locally advanced pancreatic cancer, J Oncol 2019 (2019), 9346017.
[93] J.S. Shankara Narayanan, et al., Treatment of pancreatic cancer with irreversible electroporation and intratumoral CD40 antibody stimulates systemic immune responses that inhibit liver metastasis in an orthotopic model, J Immunother Cancer 11 (1) (2023).
[94] A. Gros, et al., Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients, Nat Med 22 (4) (2016) 433–438.
[95] Y. Wang, et al., The Immunoscore system predicts prognosis after liver metastasectomy in colorectal cancer liver metastases, Cancer Immunol. Immunother. 67 (3) (2018) 435–444.
[96] T. Iwai, et al., Promising abscopal effect of combination therapy with thermal tumour ablation and intratumoural OK-432 injection in the rat osteosarcoma model, Sci. Rep. 10 (1) (2020) 9679.
[97] M.A. Postow, et al., Immunologic correlates of the abscopal effect in a patient with melanoma, N. Engl. J. Med. 366 (10) (2012) 925–931.
[98] C. O'Neill, et al., A phase 1b trial of concurrent immunotherapy and irreversible electroporation in the treatment of locally advanced pancreatic adenocarcinoma, Surgery 168 (4) (2020) 610–616.
[99] M. Lin, et al., An important discovery on combination of irreversible electroporation and allogeneic natural killer cell immunotherapy for unresectable pancreatic cancer, Oncotarget 8 (60) (2017) 101795–101807.
[100] S.M.M. Irenaeus, et al., First-in-human Study with Intratumoral Administration of a CD40 Agonistic Antibody, ADC-1013, in Advanced Solid Malignancies, Int J Cancer, 2019.