Needle-guided ablation of locally advanced pancreatic cancer: cytoreduction or immunomodulation by *in vivo* vaccination?

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Abstract: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive forms of cancer with a dismal prognosis. About a third of all patients with pancreatic cancer present with locally advanced inoperable disease and are currently designated to palliative systemic chemotherapy. Despite improved chemotherapeutic treatment by the introduction of FOLFIRINOX, median overall survival remains poor at approximately 14 months. Several needle-guided ablative therapies are investigated as treatment option for patients with locally advanced pancreatic cancer (LAPC). This review aims to give an overview of literature of the following needle-guided ablative techniques for the treatment of patients with LAPC: radiofrequency ablation (RFA), microwave ablation (MWA), cryoablation, and irreversible electroporation (IRE). The immunomodulatory effects of the ablative techniques in PDAC are also discussed. These effects in combination with immunotherapy may provide an opportunity to enhance clinical efficacy and endow local ablation with systemic tumor control. Especially less immunogenic tumors like PDAC may benefit from this new approach. Preclinical and clinical findings, possible mechanisms of action, and future research directions are addressed.

Keywords: Locally advanced pancreatic cancer (LAPC); pancreatic ductal adenocarcinoma (PDAC); radiofrequency ablation (RFA); microwave ablation (MWA); cryoablation; irreversible electroporation (IRE); immunomodulation; immunotherapy

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive forms of cancer; with a 5-year survival rate of only 8%, it is the fourth leading cause of cancer-related death in Western Europe and the United States (1). Survival has hardly improved over the past 40 years. Resection is the only potentially curative therapy, however only <20% of patients present with a tumour that is eligible for surgical resection (2). Moreover, most patients will develop disease recurrence after curative-intent surgery, resulting in a 5-year survival rate of only 12–27% (3,4) and a median overall survival (OS) of 16.8 months (5). More than 50% of patients present with metastatic disease for which systemic chemotherapy is the only proven survival prolonging treatment (6). Advances have been made for these patients with the introduction of FOLFIRINOX (combination chemotherapy containing 5-fluorouracil, leucovorin (folinic acid), irinotecan, and oxaliplatin) yielding an increase in median OS up to 11.1 months compared to 6.8 months with the prior standard-of-care gemcitabine based regimens, but at the cost of greater

Table 1 NKR-IKNL (Dutch Cancer Registry) numbers on metastatic pancreatic ductal adenocarcinoma

Patient	Overall survival (OS)	2007–2011, pre-FFX	2012–2016, post-FFX[$]	Log rank
All patients (n=3,941 and n=4,785)	Median OS	3.3 months	3.4 months	<0.001
	1-year OS	8%	10%	
Patients receiving chemotherapy (n=1,226 and n=1,758)	Median OS	5.6 months	6.4 months	<0.001
	1-year OS	15%	20%	

OS of all patients with metastatic pancreatic adenocarcinoma and those receiving chemotherapy (irrespective of type of chemotherapy) in the Netherlands between 2007 and 2016. FOLFIRINOX (FFX) became available in the Netherlands from 2012. [$], in 2015–2016: FOLFIRINOX 60%, gemcitabine & nab-paclitaxel 9.5%, gemcitabine 25%, other/unknown 5.5%.
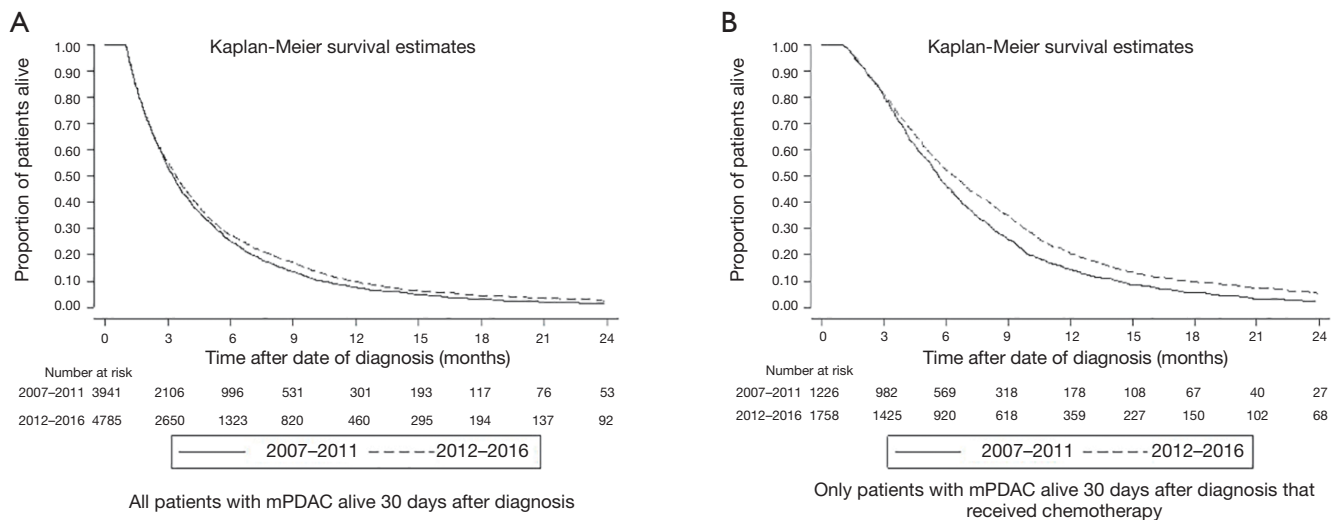

Figure 1 Kaplan-Meier survival estimates of patients with mPDAC in the Netherlands. (A) Lines show OS after date of diagnosis for all patients with mPDAC from 2007 to 2011 (straight line) representing the pre-FOLFIRINOX era and from 2012 to 2016 (dotted line) representing the time period from when FOLFIRINOX was implemented in Dutch guidelines for fit patients <75 years old [2011]. (B) Lines show OS after date of diagnosis for all patients with mPDAC that received at least 1 cycle of chemotherapy from 2007 to 2011 (straight line) representing the pre-FOLFIRINOX era and from 2012 to 2016 (dotted line) representing the time period from when FOLFIRINOX was implemented in Dutch guidelines for fit patients <75 years old [2011] (8). OS, overall survival; mPDAC, metastatic pancreatic ductal adenocarcinoma.

toxicity (7). The Dutch Cancer Registration (NKR-IKNL) shows dismal survival data (8). Median OS of all patients diagnosed with metastatic PDAC (mPDAC) from 2012 to 2016 was 3.4 months, for those patients who received at least 1 cycle of chemotherapeutic treatment median OS was 6.4 months (see *Table 1* and corresponding *Figure 1*). Nearly 30% of newly diagnosed patients with PDAC have locally advanced pancreatic carcinoma (LAPC). Due to their vascular ingrowth these tumours are considered unresectable. Patients with LAPC have a median OS of 5.1 months from the time of diagnosis (5). With the

introduction of FOLFIRINOX, survival has improved and a pooled median OS from time of diagnosis of 24.2 months was reported in a well-conducted meta-analysis but due to inclusion of retrospective reports this outcome is probably influenced by selection and immortal time bias (9). The authors state that after first-line FOLFIRINOX, almost two-thirds of patients received additional local treatments including targeted ablation, radiotherapy, chemoradiation, and surgical resection. Although no significant association was found between OS and these additional therapies, studies reporting additional therapy in more than 50%

Table 2 Key papers on needle-guided ablative cytoreduction in LAPC

Reference	Take home message
Ruarus *et al*. 2019 (13)	In this single arm prospective registered phase I/II trial, 50 patients with LAPC (of which 30% was chemo-naïve) were treated with percutaneous IRE with a median OS of 17 months. Given the study design this report provides accurate evidence on the additive beneficial effect of IRE on survival. Based on one IRE related death and half of the patients experiencing post procedural complications, percutaneous IRE should be regarded as a high-risk procedure (page 8, lines 278-281 and *Table 3*)
Månsson *et al*. 2019 (14)	No survival benefit was observed in 24 chemo-naïve patients with LAPC that were treated with primary IRE. The results emphasize the effectivity of neo-adjuvant chemotherapy in selecting patients who are likely to benefit from local treatment and does not support percutaneous IRE in the primary setting (page 8, lines 274-278 and *Table 3*)
Leen *et al*. 2018 (15)	The largest retrospective series of patients with LAPC treated with percutaneous IRE. With a median OS of 27 months the report presents the best outcomes in literature. However, given the retrospective nature of this study results should be interpreted with care (*Table 3*)
Cantore *et al*. 2012 (11)	The largest series of 128 patients with LAPC treated with open RFA. Second-line RFA proved to be superior with a median OS of 25.6 months compared to 14.7 months after primary RFA and those patients must have benefitted from a preceding treatment. Considering the morbidity rate of 28% and 2 deaths RFA for LAPC should be regarded a high-risk procedure (page 4, lines 110-120 and *Table 2*)
Song *et al*. 2014 (16)	The most recent and largest series of patients with LAPC treated with cryoablation. No significant additive benefit was achieved by cryoablation in 118 patients when combined with bypass surgery and compared to bypass surgery alone (page 5-6, lines 214-223)

LAPC, locally advanced pancreatic cancer; IRE, irreversible electroporation; RFA, radiofrequency ablation; OS, overall survival.

of patients had a better median OS (24.5 months) than studies reporting additional therapies in less than 50% of patients (17.4 months) (9). More prospective data is needed before conclusions can be drawn from this observation but it carefully appears that additional local ablative treatment after chemotherapy is beneficial in selected patients.

Overall, clinical treatment results remain poor for all stages of PDAC and for all standalone treatment modalities examined, including passive immunotherapy with monoclonal antibodies (mAbs) and active approaches employing checkpoint inhibitors, oncolytic viruses, toll-like receptor ligands, cytokines, adoptive cell transfer and peptide or dendritic cell (DC) vaccines. The discrepancy between preclinical data and clinical outcome motivates researchers to investigate novel combination strategies with the intent to enhance antigen presentation, provoke inflammation and inhibit checkpoints. In this regard, interventional radiology plays a pivotal role as tumour ablation may represent an attractive means to turn immunologically cold tumors, such as PDAC, into hot pro-immunogenic tumors.

This article addresses the following needle-guided interventional ablative modalities for the treatment of LAPC: radiofrequency ablation (RFA), microwave ablation (MWA),

cryoablation and irreversible electroporation (IRE). In May 2019 a literature search was performed in MEDLINE for available publications. Cytoreductive and immunomodulating abilities are discussed for each technique.

Cytoreduction

The rationale for local therapy in patients with LAPC is that about 30% of patients die from local progression in the absence of metastatic disease (10). Patients who do not develop metastatic disease on chemotherapeutic treatment might therefore benefit from local control of the tumor. Local ablative therapies should only be used in patients who received chemotherapy upfront and without signs of distant progression after restaging (11). This way, chemotherapy can be used to sort out patients with biologically detrimental tumor types that are prone to metastasize and who are unlikely to benefit from local therapy (12). Patients should be evaluated in a tumor board consisting of at least medical oncologists, gastroenterologists, diagnostic and interventional radiologists, radiation oncologists and surgeons. Key papers on neelde-guided ablative cytoreduction for LAPC are summarized in *Table 2*.

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RFA

RFA leverages the development of heat for tumor destruction and cytoreduction by coagulation and protein denaturation. High-frequency alternating currents are applied usually through one needle electrode that is inserted into the core of the tumor (17). Oncological results on RFA for the treatment of LAPC, remain controversial. Several studies were published evaluating the technique however, except for one study (18), all were conducted at the university of Verona Hospital Trust. Studies were mostly retrospective and considering the overlapping date ranges patient cohorts are likely coinciding to some extent, introducing selection and population bias (11,19-23). One study used a percutaneous approach (23), 6 studies reported on RFA procedures performed during open laparotomy (11,18-21,24) and one study used an endoscopic ultrasound (EUS) guided transesophageal approach (22). The largest study by Cantore *et al.* compared primary RFA combined with palliative bypass surgery to RFA as a second-line treatment subsequently to chemo(radio)therapy (11). Patients receiving primary RFA had a significantly lower median OS of 14.7 months compared to 25.6 months for those where RFA was used as secondary treatment (P=0.004). The authors explain that the difference may reflect selection bias in some patients who received second-line RFA and must have benefited from a preceding treatment, being systemic chemotherapy and/or radio-chemotherapy and/ or intra-arterial chemotherapy. In view of these scarce oncological results, the Dutch randomized-controlled PELICAN trial (NCT03690323), which compares OS data, is currently recruiting patients with non-progressive LAPC after 2 months of induction chemotherapy who are either treated with RFA plus chemotherapy or with chemotherapy alone, and might provide the ultimate answer in this matter.

A disadvantage of RFA in LAPC is the risk of collateral thermal damage considering the precarious location of the tumor near vital and delicate structures like the stomach, duodenum, bile ducts and several major vessels (25). Another drawback of RFA remains in the fact that elevation in tissue impedance, caused by excessive temperature heating, can inhibit further energy spreading through remaining tissue and may restrict the ablation zone (26). Additionally, it is challenging to maintain destructive temperatures if the ablated tumor is located near large blood vessels. This so called "heat-sink" effect occurs when thermal energy is dissipated from the ablation area by flowing blood or air, thereby decreasing RFA efficacy (27).

The most common reported serious adverse events are gastric ulcer or fistula, acute pancreatitis, pancreatic fistula, duodenal injury, biliary injury, portal vein thrombosis, and peritoneal hemorrhages. Fegrachi *et al.* reported a major complication rate of 53% using the Clavien-Dindo classification and 6% mortality in a phase II study on intraoperative RFA for LAPC (18). Roughly half of these complications consisted of delayed gastric emptying (DGE), a grade 3a complication, that was treated with endoscopic feeding tube placement. The authors claimed that if DGE had not been considered a major complication (as other centers often place preventive feeding tubes intraoperatively or simply do not interpret DGE as grade 3 complication), the major complication rate would only have been 29%. This would be in line with other literature, reporting morbidity rates ranging between 0 and 28% and 30-day mortality between 0 and 3% (11,19-24). A summary of the literature of RFA for LAPC is presented in *Table 3*.

MWA

MWA produces frictional heating by the oscillation of polar molecules, which ultimately generates coagulative tissue necrosis (28). Microwaves propagate more effectively trough different tissue types like fibrous, charred and desiccated tissues and are less affected by rapid elevation in tissue impedance than radio frequency waves (29). A drawback of the technique is that heating precision and monitoring is less exquisite compared to thermal energies that are more readily absorbed (29). Literature on the use of MWA for LAPC is scarce, probably due to the controversy concerning the possible side effects of thermal ablation for this indication. Theoretically, the complex anatomy in combination with a less predictable ablation zone does not make MWA a very attractive ablative modality for LAPC. Ierardi *et al.* reported the treatment of 5 LAPC patients with ultrasound-guided percutaneous MWA (30). The procedure was feasible in all patients and no major complications were reported. However, contrast enhanced CT-scans at one month revealed partial responses in all patients, but progressive disease in 2 patients at 4 months according to RECIST criteria. During follow-up, 2 patients died at 6 months and 1 at 10 months as result of their primary cancer that was not further specified. Carrafiello *et al.* treated 5 patients percutaneously and another 5 patients with intraoperative MWA (28). Local tumor progression rates were 37.5% at 9 months and 62.5% at 1 year, according to RECIST criteria. Minor complications were reported in 20% of the

Table 3 Oncological outcomes of radiofrequency ablation for locally advanced pancreatic cancer: retrospective studies with >15 patients and prospective studies

Reference	Study design	Patients	Median age (years)	Stage of disease/median tumor size	Approach	Morbidity	30-day mortality	Median FU (months)	Median OS (months)
Cantore et al. (2012) (11) (a)	Prospective	107	NS	LAPC/NS	Open	2.8% (n=3)	1.9% (n=2)	NS	14.7 (primary RFA); 25.6 (secondary RFA) (b)
Crinò et al. (2018) (22)	Prospective	9	67	LAPC (n=8), pancreatic metastasis (n=1)/3.6 cm	EUS-guided	0%	0%	4.3	NS
D'Onofrio et al. (2017) (23) (a)	Prospective	18	62.4 (c)	LAPC/4.8 cm	Perc.	0%	0%	NS	NS
Fegrachi (2019) (18)	Prospective	17	62	LAPC/NS	Open	53% (n=9) (d)	6% (n=1) (e)	NS	9
Frigerio (2013) (24) (a)	Retrospective	57	63	LAPC/NS	Open	14% (n=8)	0%	NS	19 (f)
Girelli (2010) (20) (a)	Prospective	50	64.5	LAPC/4.0 cm	Open	24% (n=12) (g)	2% (n=1)	8	NS
Girelli (2013) (19) (a)	Prospective	100	64 (c)	LAPC/3.6 cm	Open	26% (n=26)	3% (n=3)	12	20
Paiella (2018) (21) (a)	Retrospective	30	NS	LAPC/NS	Open	NS	0%	15	19 (h)

(a) All procedures in these studies were performed at the University of Verona Hospital Trust; given the coinciding date ranges patient data are likely overlapping; (b) RFA following any other primary treatment: radiochemotherapy, intra-arterial/systemic chemotherapy; (c) mean; (d) delayed gastric emptying (DGE) contributed for 24% to the overall complication rate; (e) cause of death was leakage of hepaticojejunostomy that was performed directly after RFA; (f) 10 out of 12 morbidities occurred in the first 25 patients, hereafter RFA temperature was decreased from 105°C to 90°C in the next 25 patients which reduced the complication rate to 8% (n=2); (g) median disease specific survival (h) inoperable PDAC was defined as distant metastasis (n=5) and/or involvement of mesenteric artery/celiac trunk and/or diffuse peritoneal infiltration. FU, follow-up; OS, overall survival; LAPC, locally advanced pancreatic cancer; NS, not specified; RFA, radiofrequency ablation; EUS, endoscopic ultrasound; Perc., percutaneous.

patients. Major events that occurred were pancreatitis and a pseudoaneurysm of the gastroduodenal artery. Lygidakis et al. treated 15 patients with histologically proven LAPC with MWA and reported on feasibility and safety of the procedure (31). No major complications or mortality occurred related to the procedure, whilst partial necrosis was achieved in all patients.

Cryoablation

On the other side of the thermal spectrum lies cryoablation. The technique utilizes argon, or nitrogen and helium gases to induce instant freezing of the tumor tissue (32). Cryoablation causes coagulative necrosis and tumor apoptosis by direct physical damage during freezing. Coinciding tissue ischemia takes place due to progressive vasoconstriction and occlusion, and endothelial damage (32).

A great advantage of cryoablation is the intraprocedural visibility of an ice ball around the tip of the cryoprobe that can be identified by ultrasound or CT and corresponds to the ablation zone. To date, 2 retrospective studies compared the safety and efficacy of cryoablation for LAPC (16,33). Li et al. compared 74 patients receiving cryoablation plus bypass surgery with 68 patients receiving only bypass surgery. Cryoablation effectively reduced tumor mass, but median OS did not significantly improve (350 versus 257 days; P=0.124) (33). Song et al. found similar results in 118 patients (5 months after cryoablation plus bypass surgery compared to 4 months after bypass surgery alone; P>0.05) (16). In both studies the post-procedural complication rates did not differ between study arms, except for increased DGE after cryoablation in the study performed by Li et al. (33). The most frequently encountered complications after intraoperative cryoablation included DGE, pancreatic or

biliary leakage, pancreatitis or gastro-intestinal bleeding or obstruction (16,33).

IRE

IRE causes irreversible nanoscale defects in the cell membrane by the application of high-voltage electric pulses, leading to cell death through apoptosis and necrosis (34,35). The technique has been investigated for several solid cancer types (36) and provides a promising alternative to thermal ablation (37). IRE is based on the pulsatile application of electric energy delivered between 2 or more electrodes that are introduced in and around the tumor (34). Due to its primarily non-thermal mechanism of action, IRE leaves supporting extracellular matrix structures unaffected, preserving the structural tissue integrity of inlaying and nearby structures like vessels and bile ducts (34). This makes the technique ideal for the ablation of diffusely growing malignancies that surround these structures, such as LAPC. Several studies have investigated the safety and efficacy of open, laparoscopic, and percutaneous IRE for LAPC. Four of these studies used IRE in selected patients also as margin accentuation after surgical resection (38-41), other studies used IRE solely as primary tumor treatment. All are summarized in *Table 4* (14,15,38-54). For primary IRE, median OS from diagnosis ranged between 10.2 and 27.0 months after a median follow-up of 1 to 29 months. The median OS from IRE treatment varied between 7.0 to 18.0 months. Some studies reported on the ability of IRE to downstage the tumor to resectability, ranging between 6.0% and 10.3% (15,43,46,47,55). Local progression after IRE varied between studies from 0 to 27.8% (14,15,38-42,44,46,47,50,52,53,55). Major complications (grade 3 or higher) varied between none (42) to 53% (51). Most frequently occurring complications were abdominal pain, nausea, anorexia, DGE or ileus, and (perforated) ulcers with or without gastro-intestinal bleeding, biliary stricture, portal vein thrombosis, bile leakage or cholangitis, pancreatic leakage, fistula or pancreatitis, and ascites. Reported mortality rates ranged from 0 to 13% and were caused by tumor progression, gastrointestinal (GI) bleeding from ulcer, portal vein thrombosis leading to liver failure, necrosis of the bile duct and duodenum, cardiopulmonary arrest, pneumonia, and pulmonary embolism. The large differences in survival outcomes between the studies may be explained by a variable time from diagnosis to IRE (range, 6.2–11.6 months), the exclusion of patients with occult metastases on diagnostic laparoscopy preceding

Table 4 Survival after primary pancreatic IRE: retrospective studies with >15 patients and prospective studies. Studies using IRE for margin accentuation in combination with surgery and case reports are excluded

Reference	Study design	Patients	Median age (years)	Stage of disease/median tumor size	Approach	Median FU (mo)	Median OS (mo)	Local recurrence	Tumor down-staging by IRE	Mortality
Belfiore et al. (2017) (42)	Retrospective	29	68.5	LAPC/NS	Perc.	29	14.0	3%	3	NS
Dunki-Jacobs et al. (2014) (41)	Retrospective	65	NS	LAPC/3.5 cm	Perc. (n=12); open (n=53)	23	NS	26%	NS	NR
Flak et al. (2019) (43)	Prospective	33	67.1	LAPC/3.0 cm (88% after chemo/radiotherapy)	Perc. (n=32); open (n=1)	9	18.5 (diagn.); 10.7 (IRE)	NS	3	7% (n=2)
Kluger et al. (2016) (44)	Retrospective	50	66.5	LAPC/3.0 cm	Open (a)	8.7	12.0 (IRE)	11%	NS	6% (n=3)
Lambert et al. (2016) (45)	Prospective	21	68.2	LAPC/3.9 cm	Open (n=19); Perc. (n=2)	NS	10.2	NS	NS	0
Leen et al. (2018) (15)	Retrospective	75	63.4	LAPC/3.5 cm (after chemo)	Perc.	11.7	27.0	38%	3	0

Table 4 (*continued*)

Table 4 *(continued)*

Reference	Study design	Patients	Median age (years)	Stage of disease/median tumor size	Approach	Median FU (mo)	Median OS (mo)	Local recurrence	Tumor down-staging by IRE	Mortality
Månsson et al. (2016) (46)	Prospective	24	65	LAPC/3.5 cm (after chemo)	Perc.	NS	17.9 (diagn.); 7.0 (IRE)	58%	2	4% (n=1)
Månsson et al. (2019) (14)	Prospective	24	68	LAPC/3.0 cm (before chemo)	Perc.	NS	13.3 (diagn.)	33%	0	0
Martin et al. (2012) (39)	Retrospective	27	61	LAPC/3.0 cm	Perc. (n=1); open (n=26) (b)	3	NS	0%	NS	4% (n=1)
Martin et al. (2013) (40)	Retrospective	54	61	LAPC/NS	Open (n=52); laparoscopic (n=2) (c)	15	20.2 (diagn.)	27.8%	NS	2% (n=1)
Martin et al. (2015) (38)	Retrospective	200	62	LAPC/2.7 cm (after chemo/radiotherapy)	Open (d)	29	24.9 (diagn.) (e)	2%	NS	2% (n=3)
Narayanan et al. (2017) (47)	Retrospective	50	62.5	LAPC/3.2±1.3 cm (e) (after chemo/radiotherapy)	Perc.	NS	27 (diagn.); 14.2 (IRE)	NS	3	6% (n=3)
Paiella et al. (2015) (48)	Prospective	10	66	LAPC/3.0 cm	Open	7.6	15.3 (diagn.); 6.4 (IRE)	NS	NS	0
Ruarus et al. (2019) (f) (13)	Prospective	50	61	LAPC (n=40) and local recurrence (n=10)/4.0 cm (68% after chemo)	Perc.	NS	17.0 (diagn.); 9.6 (IRE)	46%	0	4% (n=2)
Scheffer et al. (2017) (49)	Prospective	25	61	LAPC/4.0 cm (52% after chemo)	Perc.	12 [7–16] (g)	17.0 (diagn.); 11.0 (IRE)	NS	NS	0
Sugimoto et al. (2018) (50)	Prospective	8	64	LAPC/2.95 cm	Open/perc.	17.5	17.5 (diagn.)	38%	0	0
Vogel et al. (2017) (51)	Prospective	15	NA	LAPC/NS	Open	24	16 (diagn.)	NS	NS	13% (n=2) (h)
Yan et al. (2016) (52)	Retrospective	25	58	LAPC/4.2 cm	Open	3	NS	2%	NS	4% (n=1)
Zhang et al. (2017) (53)	Prospective	21	NA	LAPC/3.0 cm	Perc.	1	NS	NS	NS	0

(a) 24 patients underwent surgical resection and margin accentuation by IRE, 29 patients underwent primary local IRE; (b) 8 patients underwent surgical resection and margin accentuation by IRE, 19 patients underwent primary local IRE; (c) 19 patients underwent surgical resection and margin accentuation by IRE, 35 patients underwent primary local IRE; (d) 50 patients underwent surgical resection and margin accentuation by IRE, 150 patients underwent primary local IRE. Median OS from diagnosis for the surgical resection with IRE margin group was 28.3 and 23.2 months for the primary IRE group. Median OS from IRE treatment was 23 months for the surgical resection with IRE margin group and 18 months for the primary IRE group; (e) mean ± SD; (f) PANFIRE I: first 50% of inclusions were reported earlier in PANFIRE I (47); (g) median [range]; (h) one hemorrhagic gastric ulcer for which the patient refused treatment, and one after palliative exploration (progression of disease). IRE, irreversible electroporation; FU, follow-up; OS, overall survival; LAPC, locally advanced pancreatic carcinoma; Perc., percutaneous; diagn., diagnostic; NS, not specified; SD, standard deviation.

open or laparoscopic IRE (38), and differing percentages of patients who were treated with upfront chemo(radio) therapy. There is increasing evidence that neo-adjuvant chemotherapy may be an effective way to select patients who are likely to benefit from IRE (12). The recently published prospective series of Månsson *et al.* underlined this argument as it failed to demonstrate survival benefit in 24 patients with LAPC that were treated with percutaneous IRE as first line treatment (14). On the other hand, the prospective randomized PANFIRE II trial that included 50 LAPC patients of which only two-thirds received upfront chemotherapy reports a median OS of 17 months (13). The main limitation of most studies is their retrospective nature, potentially leading to selection bias. Furthermore, overlapping patient cohorts may have induced population bias. Still, the overall improved median OS of LAPC for IRE-treated patients provides an encouraging non-variable endpoint. The ongoing randomized CROSSFIRE trial aims to compare the survival benefit of IRE to stereotactic body radiotherapy in with chemotherapy pretreated LAPC patients (NCT02791503) (56).

Immunomodulation

Immunogenic effects

Besides local tumor destruction, immunomodulating properties have been identified for all four needle-guided ablative modalities. Ablation may lead to systemic tumor control through the priming or boosting of tumor-specific adaptive immunity, in effect resulting in *in vivo* vaccination (57,58). Unlike with surgical resection, after ablative therapy the treated malignancy is not removed from the body, but apoptotic or necrotic cell remnants remain available for uptake by DCs, potentially leading to effective systemic antitumor immunity (59,60). Preclinically and clinically observed abscopal effects (i.e., tumor control at distant, untreated sites) are in line with this notion (61,62). While the exact mechanism of the abscopal effect in needle-guided ablation is not fully understood, it most likely involves the activity of specialized antigen-presenting cells (APCs), the so-called DCs. DCs regulate tolerance versus immunity (63,64). Immature DCs residing in tissues are extremely proficient in antigen uptake; however, when immature, they are poor stimulators of T cells. After exposure to endogenous danger signals released by dying cells following local ablation, i.e., damage associated molecular patterns (DAMPs), DCs mature and lose their ability to take up

antigens but gain T cell stimulatory function. On the other hand, if a DC does not receive maturation signals it becomes tolerogenic, effecting the expansion of an immunosuppressive T cell subset; the regulatory T cells (Tregs) (63,64). Thus, exposure to sufficient quantities of antigens combined with maturation signals, may render DCs capable of inducing robust immune responses. Ablative tumor therapies result in massive tumor cell death, accompanied by the release of antigens, which are taken up by DCs (59,60). The release of DAMPs associated with tumor ablation properly activate the DCs, which then gain the ability to migrate to the draining lymph nodes where they may encounter and (re)activate tumor antigen-specific T cells. Subsequently, the activated tumor antigen-specific T cells may home to more distant sites in the body and eliminate (micro)metastases, thereby accounting for the aforementioned abscopal effect.

Pro-oncogenic effects

There is also increasing evidence that ablation can induce counteractive local and systemic inflammation (including cytokines IL-6 and IL-10) and pro-oncogenic growth factors (including vascular endothelial growth factor) that potentially promote aggressive tumor biology and stimulate tumor growth (65). These effects are especially seen in tumors that are treated sub-lethally. Thermally (heat or cold) ablated lesions can be divided into two zones (26). The central zone, directly next to the thermal source, is characterized by tissue coagulation and cell necrosis. These necrotic cells release DAMPs that mature DCs, thus effecting immunogenic cell death, resulting in tumor-specific immunity. The peripheral or translational zone is characterized by sub-lethal hyper- or hypo-thermia that induces cell apoptosis. These apoptotic cells may be cleared by macrophages, thus leaving insufficient DAMPs and antigens for activation and antigen uptake by DCs (an immunologically "silent" form of cell death). Immature DCs can trigger immunosuppressive signals that lead to T cell inactivation and immune tolerance (66,67). Clinical evidence for pro-oncogenic or so called off target effects of ablation exists for colorectal liver metastases, hepatocellular carcinoma, and renal tumors and has been linked to a higher rate of distant tumor development and overall worse patient outcomes (68-70). It is not yet fully understood how to induce immunogenic effects while avoiding pro-oncogenic effects. Different ablation techniques but also varying ablation parameters for the same technique might

Table 5 Key papers on needle-guided ablative immunomodulation in PDAC

Reference	Take home message
Shao *et al.* 2019 (78)	*In vitro* experiment demonstrating that IRE induces the most pronounced release of proteins/antigens, followed by cryoablation. Although cryoablation releases the most "not denatured" proteins, IRE outperforms both cryo- and heat-ablation in terms of T cell activation. This study demonstrates that despite each local therapy's ability to destroy cells, IRE most potently triggers immunogenic cell death and subsequent T cell induction (page 10-11, lines 505-518)
Giardino *et al.* 2017 (79)	In 10 patients with LAPC, treated with open RFA, 3 days after treatment a downregulation of all leukocyte populations was observed. Tregs remained low after treatment which could be attributed to the RFA treatment. Also, CD4^{+} and CD8^{+} T-cell populations increased significantly after 30 days which could imply the activation of an adaptive T cell response (page 10, lines 413-434)
Scheffer *et al.* 2019 (80)	In 10 patients with LAPC, treated with percutaneous IRE, 2 weeks after treatment an upregulation of PD-1 on both CD4^{+} and CD8^{+} T cells was observed together with a downregulation of Tregs. Simultaneously a tumor antigen specific T cell response was observed that was related to a better OS. This result suggests an immunogenic period after IRE that might be further leveraged by immunotherapy with PD-1 checkpoint blockade (page 11, lines 537-557)
Pandit *et al.* 2019 (81)	In 11 patients with LAPC treated with open IRE several Treg subsets were observed to remain low, 3-5 days after treatment, while a steady increase in Tregs was observed after pancreatectomy. These findings suggest that IRE might alleviate immunosuppression in patients with LAPC and implies a rationale for combined treatment with immune enhancing drugs (page 11, lines 526-536)
Zhao *et al.* 2019 (82)	Demonstration of the synergistic effects of IRE with anti-PD-1 mAbs in an orthotopic murine pancreatic cancer model. The combination therapy significantly promoted CD8^{+} T cell infiltration to the tumor without recruiting other immunosuppressive cells. A durable memory T cell response was achieved with a striking cure rate of 36–43% (page 11, lines 558-566)

LAPC, locally advanced pancreatic cancer; IRE, irreversible electroporation; RFA, radiofrequency ablation; mAbs, monoclonal antibodies.

have opposite effects in the same tumor environment. Future research exploring the immunogenic effects of ablation should take the potential pro-oncogenic effects into consideration.

The pancreatic immune escape

Generally, PDAC is regarded as weakly immunogenic (71). This low immunogenic potential may in part be due to a lack of spontaneous cell death and collateral antigen release by cancer cells (72). Priming of tumor-specific T cells in PDAC is further hampered by active immune suppression perpetrated by the tumor (73). Recent reports have shown that the prevalence of suppressive Tregs is increased in several solid tumors, particularly in the tumor microenvironment (74). Data from Hiraoka *et al.* confirmed that Tregs indeed play a role in controlling the immune response in PDAC from the premalignant stage to established cancer (75). The prevalence of Tregs was significantly increased in the stroma of invasive PDAC compared with the stroma of non-neoplastic inflammation of the pancreas. Another important immunosuppressive subset consists of myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immature

myeloid cells that are increased in cancer patients and converted to highly suppressive cells (76). Gabitass *et al.* demonstrated a significant increase in circulating MDSCs in patients with PDAC which correlated with an elevation in Tregs and was an independent prognostic factor (77). Key papers on needle-guided ablative immunomodulation fin PDAC are summarized in *Table 5*.

Evidence of ablative immunomodulation in PDAC

RFA

The ability of RFA to modulate the immune system and to induce anti-tumor immune responses has been demonstrated in several tumor types, such as hepatocellular carcinoma (83), colorectal and other metastatic liver tumors (77,84) and lung carcinoma (85). However, evidence for the advantageous immunomodulatory effects of RFA in PDAC remains scarce. RFA causes protein denaturation, cell necrosis, and vessel disruption through hyperthermia. The heat upregulates heat shock protein 70 (HSP-70) (86) that plays an important role in the activation of naive DCs (59). RFA can thus raise the frequency of activated DCs in PDAC leading to an adaptive T-cell response (87). Giardino *et al.* published the only clinical data on the influence of

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RFA for PDAC on the immune system (79). Peripheral blood samples of 10 patients with LAPC showed a systemic immune response after intra-operative RFA, which was attributed to a possible transitional zone of apoptosis-undergoing tumor tissue exposing tumor-specific antigens. Immediately following RFA (day 3) a downregulation of all leukocytic populations was observed, which can be either due to surgically-induced immunosuppression or the RFA treatment (88). After 30 days, CD4^{+} and CD8^{+} T-cell populations increased significantly compared to day 3, implying the activation of an adaptive T-cell response. However, there was no significant difference between pre-RFA (day 0) and frequencies at day 30, raising the question whether the CD4^{+} and CD8^{+} T cells had simply returned to baseline after being downregulated at day 3. Immunosuppressive Tregs remained stable 3 days after RFA, which was considered remarkable since laparotomy and heating could have favored their expansion. The authors speculate that RFA downregulates these immunosuppressive cells (79).

MWA

The first clinical immunomodulatory effects of MWA were found in patients with prostate cancer, where a transient increase in CD4^{+}/CD8^{+} ratio was observed most severely at 2 months after ablation (89). Since MWA is often used for liver tumors, multiple studies investigating immunomodulation regarding this ablative modality have been performed in this organ. Increased infiltration rates of immune stimulatory cells like CD4^{+} T cells, CD8^{+} T cells, monocytes, NK cells were observed in biopsies of the ablation zone (90,91) and increased levels of CD3^{+}, CD4^{+} and IL-12 were found in peripheral blood up to 30 days after the treatment whilst the immunosuppressive cytokines IL-4 and IL-10 decreased significantly (92). Most strikingly an increased OS and decreased recurrence rate were both associated with a high extent of immune cell infiltration (93). Studies investigating the immune response related to MWA for PDAC have yet to be performed.

Cryoablation

Of all needle-guided ablation methods the influence of cryoablation on the immune system has been studied most extensively and evidence for both immunostimulatory (94,95) and immunosuppressive (96) responses has been published suggesting that the extent to which the effects

occur is subject to several factors like organ type, tumor type and ablation parameters. Nevertheless, robust preclinical work demonstrated that cryoablation induces an effective DAMP release and antigen depot resulting in maturation of DCs in a B16 mouse model. This leads to tumor-specific immune responses that protected half of the ablated mice against tumor challenge (59). *In vitro* results comparing the quality and quantity of proteins released by cryoablation versus heat-based ablation showed that cryoablation outperformed heat in terms of total protein release and of native (not denatured) protein release ensuing more effective priming of the immune system (78). The immunomodulatory effects have also been compared in the peripheral blood samples of patients with different tumor types by Takaki *et al*. The authors reported no change in Tregs, T1 or T2 helper cells before and after ablation for both modalities. However, there was an elevation of CD8^{+} T cells after heat-based ablation, but none was observed after cryoablation leading to the authors conclusion that heat-based ablation might be more effective to enhance systemic anti-tumor immunity (97). Clinical evidence of the immunomodulatory effects of cryoablation in PDAC is however nonexistent.

IRE

Two characteristics of IRE potentially make the technique a good candidate for the induction of a systemic antitumor immune response in PDAC. IRE-induced cell death and reduced tumor load, may lead to a simultaneous release of immunogenic tumor cell remnants and a reduction in tumor-associated immunosuppression. Secondly, because the larger vessels remain intact, APCs like DCs, with the ability to induce an immune response against the released tumor antigens, as well as subsequently primed and recruited effector T cells, should be able to infiltrate the lesion (34,98). Vessel patency could represent a great advantage compared to thermal ablation which destroys microvasculature. This was demonstrated by Bulvik *et al*. in an animal model, where leukocyte infiltration into the ablation zone was present in IRE-treated, but not in RFA-treated lesions (62).

The ability of IRE to induce immunogenic cell death has been investigated *in vitro* in B16 melanoma cell lysates and was compared to that of cryotherapy and heat-based ablation (78). After viability assessment cell lysates were collected and assessed for protein release, protein denaturation, antigen release, and T cell activation (antigen-

specific CD8$^+$ T cell proliferation). Results showed that IRE induced the most pronounced release of proteins/antigens, followed by cryoablation. Although cryoablation released the most "not denatured" proteins, IRE outperformed both cryo- and heat-ablation in terms of T cell activation. This study demonstrates that despite each local therapy's ability to destroy cells, IRE most potently triggers immunogenic cell death and subsequent T cell induction (78).

In vivo evidence underlines this observation. In mice bearing implanted pancreatic tumors, IRE induced an increase in infiltrating macrophages and T cells that was 2 times greater compared to cryoablation at 6 hours, 12 hours and 24 hours after treatment (99).

To date 2 groups published clinical data about the immunomodulatory effects of IRE in patients with LAPC. Pandit *et al*. analyzed blood samples by flow cytometry of 15 patients for 3 clinically correlated Treg populations (81). In this study one group underwent intraoperative IRE (n=11) and the other group (n=4) underwent a pancreatectomy. Blood was drawn before treatment, 1 day after treatment and between 3 and 5 days after treatment. In both groups, all Treg subsets decreased 1 day after treatment followed by a steady increase in the pancreatectomy group, whereas Treg levels remained low in the IRE group between day 3 and day 5. This suggests that IRE may alleviate immunosuppression in LAPC.

Scheffer *et al*. performed flowcytometric profiling on the blood of 10 patients with LAPC who were treated with percutaneous IRE. Blood was collected pre-treatment, 2 weeks and 3 months after IRE (80). Frequencies and activation states of several immune subsets were analyzed, including CD4$^+$ T cells, CD8$^+$ T cells, Tregs, conventional and plasmacytoid DCs, monocytes, and MDSCs. At 2 weeks post IRE a transient decrease in systemic Treg rates and a simultaneous transient increase in activated PD-1$^+$ T cells was found, consistent with the temporary reduction of tumor-related immunosuppression after IRE. The authors also found post-IRE boosting of a pre-existing Wilms tumor 1 (WT1) antigen, expressed by 75% of pancreatic tumors (100), specific T cell response by IFN-γ Elispot read-out in 2 out of 3 patients as well as a de novo induction of these responses in another 2 patients. There was a trend for these WT1 T cell responses to be related to longer OS (P=0.055). These findings demonstrate a systemic and tumor-specific immune stimulatory effect of IRE and in addition to the results of Pandit *et al*. show that the effect endures for at least 2 weeks.

Elaborating on these observations Zhao *et al*.

demonstrated in an orthotopic murine pancreatic cancer model that IRE combined with checkpoint inhibition with anti-PD-1 mAbs promoted tumor infiltration by CD8$^+$ cytotoxic T cells without recruiting other immunosuppressive cells to the tumor. This treatment combination significantly prolonged survival. Most strikingly, the IRE + anti-PD-1 treatment achieved a cure rate of 36–43% with a durable memory T cell response (82).

Future perspectives

Cancer immunotherapy is based on the premise that tumors can be recognized as foreign and can be effectively attacked by an activated immune system. Efficacy of immunotherapy in PDAC remains limited, probably due to this tumor type's immunosuppressive microenvironment. Local ablation techniques may affect both the immunogenic release of pancreatic tumor-derived antigens and the alleviation of tumor-associated immunosuppression, thus achieving *in vivo* vaccination against the tumor.

The clinical findings presented by Giardino *et al*., Pandit *et al*., and Scheffer *et al*. and the preclinical work published by Zhao *et al*. support the combination of pancreatic tumor ablation with therapeutic immunomodulation (79-82). Ablation may provide a window of opportunity for potentiating the clinical efficacy of immunotherapy. In particular, the observation of transiently increased PD-1 expression on T cells after IRE in patients with LAPC (80) and the durable memory T cell response achieved in mice (82) point towards IRE as a particularly powerful technique in this regard. The studies strongly support the combined application of IRE with check point inhibition for PDAC (see *Figure 2* for a research setup example combining IRE ablation with intratumoral and/or systemic immunotherapy).

Several factors concerning the combination of ablation and immunotherapy remain uncertain. Especially the timing and sequence of ablation and the application of immune stimulatory drugs seem to be of importance and warrant further investigation (101).

Conclusions

This review gave an overview of the currently available literature on needle-guided ablative techniques for the treatment of patients with LAPC and their effects on the immune system. In general, the safety profile of pancreatic ablation is defined as acceptable. However, major adverse
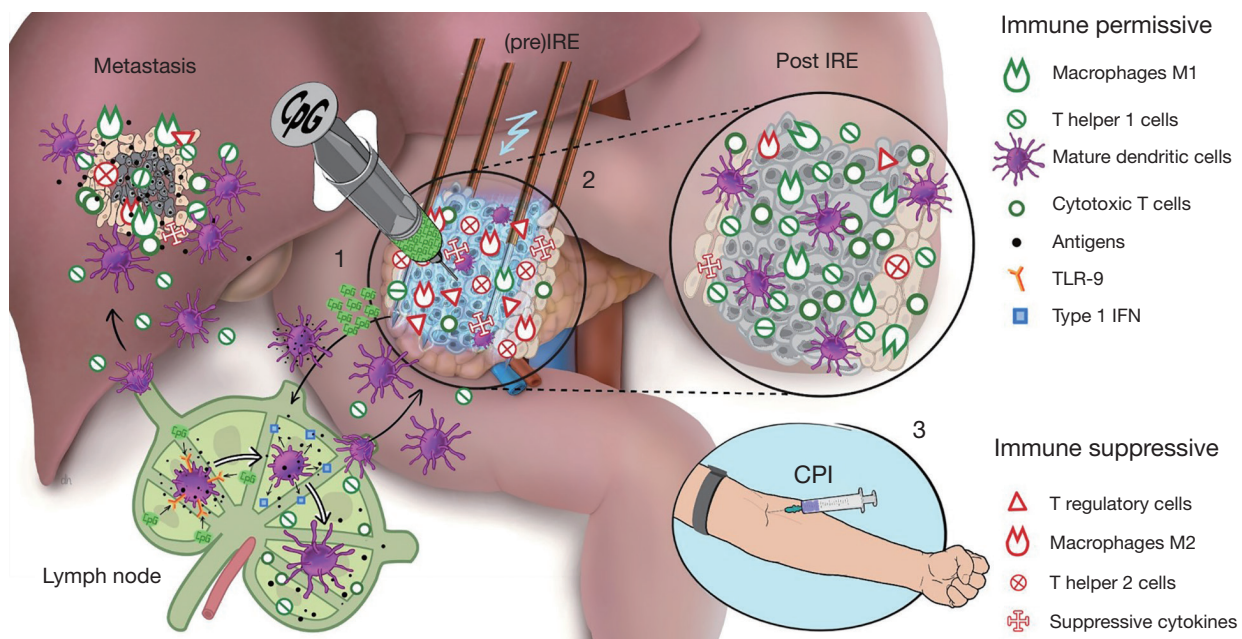
Figure 2 Electro immunotherapy. The primary pancreatic tumor maintains an immunosuppressive microenvironment by the presence of Tregs, M2 Macrophages, T helper 2 cells and suppressive cytokines (red icons) and downregulation of effector T cells. 1: CpG is injected in the primary tumor, it diffuses to the primary draining lymph nodes where it binds toll like receptor 9 (orange receptor) on immature dendritic cells causing them to mature (purple cells), this results in a type-1 IFN (blue icons) release and activation of cytotoxic and t helper 1 cells (green icons). 2: IRE of the primary pancreatic tumor causes massive cell death resulting in release of antigens (black dots) that are taken up by dendritic cells and transported to the lymph node where an adaptive anti-tumor specific T cell response can be elicited (green icons). As IRE reduces the tumor mass it also leads to a reduction in the secretion of immunosuppressive Tregs, MDSCs and cytokines. The tumor microenvironment's shift in balance from immunosuppressive (red icons) to immune permissive (green icons) is illustrated in the post-IRE enlargement. 3: Intra venous injection with checkpoint inhibition (CPI). PD-L1 on the cancer cell surface binds to PD-1 on an immune cell surface, which inhibits immune cell activity. Checkpoint inhibitors bind checkpoint receptors on the tumor cell (PD-L1) or T cell (PD-1) and therefore block this regulatory interaction and allow the T cells to attack the tumor cells (not illustrated). The combination of all 3 treatment modalities (IRE, CpG, CPI) may work synergistically and together alter the tumor microenvironment and induce a systemic immune response to ultimately cause an abscopal effect in distant metastatic lesions (illustrated in the liver). IRE, irreversible electroporation; MDSCs, myeloid-derived suppressor cells.

events do occur and even mortality has been reported for all ablative techniques. High-quality evidence for survival benefit for any local ablative modality over chemotherapy alone is scarce. Nevertheless, the promising survival in several studies where effective chemo(radio)therapy preceded ablation, warrants completion of the ongoing randomized controlled phase III trials to provide closure on needle-guided ablation's additive value. Emerging preclinical and clinical data on the capability of ablation to induce *in vivo* vaccination should motivate researchers to design smart studies with combinations of local ablation and immunotherapies to investigate their efficacy and to fill remaining knowledge gaps concerning timing and sequencing. Effective combination of ablation and immunotherapy could hypothetically turn the immune suppressed tumor microenvironment of PDAC into an immune permissive environment and ultimately result in a longer lasting survival benefit for patients.

Footnote

Conflicts of Interest: MR Meijerink is a paid consultant for AngioDynamics. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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