Irreversible electroporation in pancreatic cancer

Past experience, present challenges and future perspectives

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BY
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DENMARK

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Flak RV, Stender MT, Stenholt L, Thorlacius-Ussing O, Petersen LJ (2020). Imaging Response Evaluation after Local Ablative Treatments in Locally Advanced Pancreatic Cancer: An Expedited Systematic Review. HPB 22: 1083–91. https://doi.org/10.1016/j.hpb.2020.04.011.

Flak RV, Stender MT, Jensen TM, Andersen KL, Henriksen SD, Mortensen PB, et al (2019). Treatment of Locally Advanced Pancreatic Cancer with Irreversible Electroporation – a Danish Single Center Study of Safety and Feasibility. Scand J Gastroenterol 54: 252–8. https://doi.org/10.1080/00365521.2019.1575465.

Laursen RV (written under former name), Hansen SB, Risager MB, Omland Ø (2013). [Prevalence of Methicillin Resistant Staphylococcus Aureus Is Increasing in Danish Farming]. Ugeskr Laeger 175: 2243–6.

ENGLISH SUMMARY

Pancreatic cancer (PC) is a detrimental disease with few treatment options for most patients. The global incidence is rising, and advances in treatment are merely incremental when compared to those of other cancers. Local treatment with irreversible electroporation (IRE) has been proposed as a treatment option in approximately 30% of patients with nonmetastatic but unresectable disease at diagnosis, i.e., locally advanced PC (LAPC).

In the current thesis, four studies are presented that aim to investigate the feasibility and safety of IRE, identify current challenges in evaluating the efficacy of IRE based on imaging and evaluate the feasibility of combining IRE and chemotherapy.

The first study is a prospective clinical trial of patients with LAPC. The study found an overall adverse event rate of 53%, with a major complication occurring in 20% of ablations(1). The median overall survival was 10.7 months after IRE treatment, with a two-year survival rate of 25%. Retreatment with IRE and larger baseline tumor size were identified as potential risk factors for severe adverse events and poorer survival. A review of the published literature was not able to definitively establish the efficacy of IRE, but preliminary evidence is encouraging.

The second study is a systematic literature review conducted to examine the knowledge and evidence regarding imaging response evaluation after ablative therapies in LAPC treatment(2). The study included 34 papers and found a marked difference in the methodology and reporting of key imaging parameters, e.g., the target for evaluation (lesion level or patient level) and the timing and methods used. Statistical testing of the correlation between imaging outcomes and survival was performed in only one study. Based on the heterogeneity of the included papers, several recommendations for future trials were made to facilitate cross comparison and meta-analysis of results.

The third study is a prospective cohort study of all PC patients treated with IRE in our institution(3). The study aimed to correlate imaging outcome categories based on functional and morphological scans to survival after the intervention. The study found that patient-level outcomes were correlated with survival, whereas lesion-level outcomes were not. Several trends were, however, noticed, suggesting that differences in some lesion-level outcomes would become significant in a larger cohort. Functional imaging was not superior to morphological imaging, most likely due to the results being confounded by inflammation.

The fourth study is an animal experimental trial aiming to characterize the ablation zone after IRE and evaluate the existence of a proposed zone of reversible electroporation (RE) in conjunction with the IRE zone (Appendix 4). In extension, the

study aimed to examine the feasibility of combining IRE and chemotherapy to elicit a synergistic electrochemotherapy-like effect. The study found a marked retention of magnetic resonance contrast in the penumbra of the IRE ablation zones, which is consistent with theoretical models and with the findings of the single existing paper on the subject. However, the study failed to demonstrate that bleomycin, a chemotherapeutic agent commonly used in electrochemotherapy, was captured in the RE zone.

In conclusion, several important and possibly lethal complications have been seen after IRE in LAPC, but most are manageable. The efficacy and effect size of IRE in LAPC is not definitely established, but randomized controlled studies are being conducted. Imaging response evaluation after ablative treatments in nonmetastatic PC is a difficult endeavor with little evidence or consensus. Patient-level outcomes correlate with survival, and several promising lesion-level outcomes warrant further investigation. IRE may have a synergistic effect with chemotherapy, but the timing and choice of chemotherapeutic agents are critical.

DANSK RESUME

Pancreascancer (PC) er en alvorlig kræftform med få behandlingsmuligheder for størstedelen af patienterne. Den globale incidens er stigende og udviklingen i nye behandling af sygdommen er uden store landvindinger, i modsætning til mange andre kræftformer. Lokal behandling med irreversibel elektroporation (IRE) er blevet foreslået som en behandlingsmulighed til de ca. 30 % af patienterne som har ikke-metastatisk og ikke-resektabel sygdom på diagnosetidspunktet (lokalavanceret PC (LAPC)).

I denne afhandling præsenteres fire studier. Målet med studierne er at undersøge gennemførligheden og sikkerheden af IRE, at identificere aktuelle udfordringer i forbindelse med vurdering af effekten af behandlingen vha. billeddiagnostik og evaluere gennemførligheden af kombineret IRE og kemoterapi.

Studie 1 er et prospektivt studie af IRE til behandling af patienter med LAPC(1). Studiet viser at der opstod uønskede hændelser efter 53 % af behandlingerne. Hos 20 % opstod der alvorlige hændelser. Medianoverlevelsen efter IRE var 10.7 måneder, og 25 % var i live efter to år. Genbehandling med IRE og stor tumorstørrelse ved baseline blev identificeret som mulige risikofaktorer for uønskede hændelser og for dårligere overlevelse efter behandlingen. Effektstørrelsen og effektiviteten kan ikke endeligt vurderes ud fra de studier som er udgivet til dato. De præliminære resultater vedrørende effektivitet er lovende.

Studie 2 er en systematisk litteraturgennemgang med formålet at gennemgå evidensen for billeddiagnostisk responsevaluering efter ablations-behandlinger til LAPC(2). Studiet inkluderede 34 artikler og viser at metoderne og rapporteringen af vigtige billeddiagnostiske endepunkter er yderst heterogen. Kun et enkelt studie udførte statistiske analyse af korrelationen mellem billeddiagnostisk endepunkter og overlevelse efter behandlingen. Baseret på heterogeniteten af de inkluderede studier fremføres adskillige forslag til forbedring og ensartning af metoderne for at facilitere sammenligning og metaanalyse af resultater.

Studie 3 er et prospektivt studie af alle IRE-behandlede PC patienter fra vores institution(3). Studiets formål var at undersøge korrelationen mellem billeddiagnostiske responskategorier, baseret på funktionelle og morfologiske skanninger, og overlevelsestiden efter interventionen. Studiet fandt at responsevaluering foretaget på patientniveau er korreleret med overlevelsestiden, hvorimod responsevaluering foretaget på læsionsniveau ikke er. Flere af endepunkterne på læsionsniveau tenderede imod signifikante resultater, hvilket tyder på at disse vil blive signifikante i en større undersøgelse. Funktionel billeddiagnostik var ikke bedre end konventionel morfologiske billeddiagnostisk, hvilket, højst sandsynligt, skyldes inflammation.

Studie 4 er et dyreeksperimentelt studie med formålet at karakterisere ablationszonen efter IRE og bedømme eksistensen af en formodet reversibelt elektroporeret zone i periferien af IRE ablationer (Appendix 4). I forlængelse heraf var formålet at undersøge om denne zone kan udnyttes til at opnå en synergetisk effekt imellem IRE og kemoterapi. Studiet fandt en tydelig retention af magnetisk resonans kontraststof i en penumbra rundt om IRE-zonen, hvilket stemmer overens med de matematiske modeller og det eneste andet billeddiagnostisk studie, som, til dato, har beskæftiget sig med emnet. Det var ikke muligt at påvise retention af bleomycin i den pågældende zone, hvilket er overraskende.

Konklusioner er, at der er observeret flere vigtige og potentielt livstruende komplikationer efter IRE-behandling af LAPC, men at de fleste kan håndteres. Effektiviteten og effektstørrelsen af IRE til behandling af LAPC er ikke definitivt etableret, men randomiserede studier er på vej. Billeddiagnostisk responsevaluering efter ablationsbehandling af PC er svært og næsten uden evidens eller konsensus. Responskategorier på patientniveau korrelerer med overlevelsestiden og flere kategorier på læsionsniveau er lovende og bør undersøges nærmere. Det er sandsynligt at IRE har en synergetisk effekt med kemoterapi, men timing og valg af kemoterapi er vigtig for at opnå den ønskede effekt.

This PhD thesis is based on the following papers:

Flak RV, Stender MT, Jensen TM, Andersen KL, Henriksen SD, Mortensen PB, et al (2019). *Treatment of Locally Advanced Pancreatic Cancer with Irreversible Electroporation - a Danish Single Center Study of Safety and Feasibility*. Scand J Gastroenterol: 54(2):252-258.

Flak RV, Stender MT, Stenholt L, Thorlacius-Ussing O, Pedersen LJ (2020). *Imaging Response Evaluation after Local Ablative Treatments in Locally Advanced Pancreatic Cancer: An Expedited Systematic Review*. HPB: 22(8):1083-1091.

Flak RV, Fisker RV, Bruun NH, Stender MT, Thorlacius-Ussing O, Petersen LJ (2021). *Usefulness of Imaging Response Assessment after Irreversible Electroporation of Localized Pancreatic Cancer - Results from a Prospective Cohort.* Cancers (Basel): 13: 2862.

Flak RV, Hansen ACN, Nielsen MS, Kjærgaard B, Thorlacius-Ussing O. *A Characterization of the Reversibly Electroporated Penumbra During Irreversible Electroporation - Results from an Animal Experimental Trial*. (Article in draft)

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ABBREVIATIONS

^{18}F-FDG	Fluorodeoxyglucose (^{18}F)
AJCC	American Joint Committee on Cancer
App.	Appendix
ASA	American Society of Anesthesiologists
BRPC	Borderline resectable pancreatic cancer
BSA	Body surface area
CA	Celiac artery
CA-19-9	Carbohydrate antigen 19-9
CaEP	Calcium electroporation
CAR	Celiac artery resection
CBD	Common bile duct
CD	Clavien–Dindo
CHA	Common hepatic artery
CHD	Common hepatic duct
CONSORT	Consolidated Standards of Reporting Trials
CPI	Checkpoint inhibitor
CR	Complete response
CT	Computed tomography
CTP	Cryothermal probe ablation
DNA	Deoxyribonucleic acid
ECOG	Eastern Cooperative Oncology Group
ECT	Electrochemotherapy
EORTC	European Organization for the Research and Treatment of Cancer
ESOPE	European Standard Operating Procedure for Electrochemotherapy
FOLFIRINOX	Folinic acid (leucovorin) + fluorouracil + irinotecan + oxaliplatin
GDA	Gastroduodenal artery
GET	Gene electrotransfer
GI	Gastrointestinal
H-FIRE	High-frequency irreversible electroporation
HA	Hepatic artery
HIFU	High-intensity focus ultrasound
HU	Hounsfield units
i.t.	Intratumoral
i.v.	Intravenous
IMRT	Intensity modulated radiation therapy
IPMN	Intraductal papillary mucinous neoplasms
IRE	Irreversible electroporation
IVC	Inferior vena cava
LAPC	Locally advanced pancreatic cancer
MCN	Mucinous cystic neoplasia
MDT	Multidisciplinary team

MR	Mortality rate
mRECIST	Modified response evaluation criteria in solid tumors
MRI	Magnetic resonance imaging
MRR	Mortality rate ratio
MTV	Metabolic tumor volume
MWA	Microwave ablation
NCCN	National Comprehensive Cancer Network
NET	Neuroendocrine tumors
NICE	National Institute for Health and Care Excellence
NPV	Nonperfused volume
NSA	Number of signal averaging
NSAID	Nonsteroidal anti-inflammatory drug
OR	Objective response
OS	Overall survival
PanIN	Pancreatic intraductal neoplasia
PC	Pancreatic cancer
Pd	Pancreatic duct
PD	Progressive disease
PDAC	Pancreatic ductal adenocarcinoma
PEF	Pulsed electrical fields
PERCIST	Positron emission tomography response criteria in solid tumors
PET	Positron emission tomography
PFS	Progression-free survival
PR	Partial response
PRISMA	Preferred Reporting Items for Systematic Reviews and Metanalyses
PS	Performance status
PV	Portal vein
RCT	Randomized controlled trial
RE	Reversible electroporation
RECIST	Response evaluation criteria in solid tumors
RFA	Radiofrequency ablation
SBRT	Stereotactic body radiation therapy
SD	Stable disease
SEMS	Self-expanding metal stents
SIR	Society of Interventional Radiologists
SMA	Superior mesenteric artery
SMV	Superior mesenteric vein
SUV_{max}	Maximum standard uptake value
SUV_{mean}	Mean standard uptake value
TLG	Total lesion glycolysis
TOF	Train of four monitoring
US	Ultrasound
WHO	World Health Organization

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CHAPTER 1. INTRODUCTION

Pancreatic cancer (PC) is a devastating disease with a rising incidence. While the prognosis of most other gastrointestinal cancers has greatly improved during the past decades, PC survival remains dismal. At the same time, the incidence is rising(4).

The only therapy that can provide a potential cure is radical surgery. However, only approximately 15-20% of patients are eligible for resection at diagnosis(5), and the majority of patients will experience recurrence even after apparent radical resection(6). It is evident that there is great need for innovations to improve survival in the large group of patients with metastatic or unresectable local disease.

This PhD thesis will explore several aspects of a novel therapy option that has been introduced within the past decade, irreversible electroporation (IRE). The overall aim of this thesis is to examine the evidence for IRE in locally advanced PC (LAPC) treatment, to identify important knowledge gaps and to explore the future perspectives of IRE in PC.

CHAPTER 2. BACKGROUND

2.1. GROSS ANATOMY AND PHYSIOLOGY OF THE PANCREAS

The pancreas is a 12-15 cm long organ residing in the retroperitoneal space behind the stomach and the lesser peritoneal sack. It is an essential part of the digestive system as well as an important endocrine organ.

The pancreas is anatomically divided into four sections: the head, body, tail and uncinate process (Figure 1). The head of the pancreas is situated in the curve of the duodenum. The neck of the pancreas is defined by the groove created by the superior mesenteric vein (SMV) and the superior mesenteric artery (SMA). Likewise, the uncinate process is the part of the pancreatic head that lies posterior to the SMA/SMV. The tail is the most distal part of the organ, residing near the spleen. The body is the part between the neck and the tail.

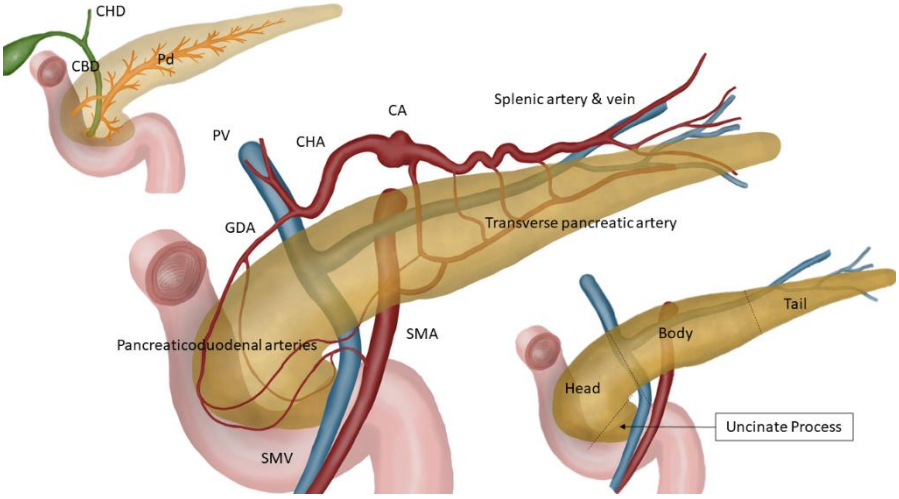


Figure 1: Gross anatomy of the pancreas

CHD = Common hepatic duct, CBD = Common bile duct, Pd = Pancreatic duct, PV = portal vein, CHA = Common hepatic artery, CA = Celiac artery, GDA = Gastroduodenal artery, SMA = Superior mesenteric artery, SMV = Superior mesenteric vein.

Microscopically, the pancreas consists primarily of exocrine tissue with cells forming acini and ducts. The ducts form intralobular ducts that, in turn, form interlobular ducts and finally the main pancreatic duct (Pd). The Pd drains the produced enzymes into the duodenum where the enzymes are activated and the breakdown of proteins, fats and carbohydrates for digestion begins. Scattered within the exocrine tissue are Langerhans islets, which consist of clusters of endocrine cells producing several hormones, most importantly insulin and glucagon. The common bile duct (CBD) passes through the head of the pancreas and conjoins with the Pd just before entering the duodenum.

The blood supply for the pancreas is complex. The body and tail of the pancreas are supplied by several branches from the splenic artery, ultimately forming the transverse pancreatic artery. The head of the pancreas is primarily supplied by two branches of the gastroduodenal artery (GDA), the anterior and posterior pancreaticoduodenal arteries. These arteries also receive some blood from the SMA.

2.2. PANCREATIC CANCER

Globally, approximately 495,000 new cases of PC were reported in 2020, making PC the 12th most common cancer according to the Global Cancer Observatory(7). However, despite global research efforts and advances in treatment, 466,000 people died from the disease the same year, making it the 7th deadliest cancer in terms of absolute death toll. It has been projected that PC will become the second leading cause of cancer death in the USA within the next twenty to thirty years(5). The current incidence rate of PC in Denmark is approximately 900-1000 cases/year, with a five-year survival rate of 8%(8) and a median age at diagnosis of 70 years(9).

2.2.1. SYMPTOMATOLOGY AND DIAGNOSIS

One of the main challenges in the diagnosis of PC is that symptoms can be vague and unspecific, especially in early disease stages, which leads to delayed diagnosis and thus more advanced disease in most patients. Symptoms include abdominal pain, abnormal liver function test results, jaundice, new-onset diabetes, dyspepsia, nausea and vomiting, back pain and weight loss, in the order of frequency(10).

Most pancreatic cancers are discovered by computed tomography (CT) scans and confirmed by histopathological examination of biopsies. The road from clinical suspicion to diagnosis can follow three different pathways in Denmark: 1) following a CT scan performed due to clinical signs of PC as a part of the Danish National Integrated Cancer Pathway for PC(11); 2) following a CT and/or positron emission tomography (PET) scan performed as part of a generalized cancer diagnostic approach; or 3) as an incidental finding on imaging procedures performed for other

reasons. Biopsies to confirm the diagnosis of PC are performed using endoscopic ultrasound guidance in most cases but are not required prior to surgery in clear-cut cases if radical resection is feasible without neoadjuvant therapy(12). In the latter case, the diagnosis is confirmed after excision.

To date, no health care system in the world screens for PC in patients without known cancer inheritance syndromes or known cystic pancreatic lesions, but knowledge regarding the genetic/proteomic profiles of PC patients is advancing and may provide viable screening tools in the future(13).

2.2.2. RISK FACTORS

There are many risk factors for PC, but in contrast to the strong causal relationship between smoking and lung cancer, no single lifestyle factor carries a very high relative risk of pancreatic malignancy. Smoking is associated with an odds ratio for PC of 1.2 for former smokers and 2.2 for current smokers(14). Chronic pancreatitis, comparatively, carries a much higher risk of developing PC (odds ratio 2.7-13.6) but is associated with only approximately 1% of cases(15). A few rare inherited genetic disorders are highly correlated with PC but are associated with only 5-10% of cases globally(16,17). The most common inherited disorders associated with PC are listed in Table 1.

Inherited syndromes	**Susceptibility gene/chromosomal mutation region**	**Increased risk of pancreas cancer**
Hereditary pancreatitis	*PRSS1* (7q35)	50- to 80-fold
Hereditary nonpolyposis colorectal cancer (HNPCC)	*h*MSH2, *h*MSH1, *h*PMS2, *h*MSH3, *h*PMS1, *h*MSH6/GTBP	Undefined ↑risk
Hereditary breast and ovarian cancer	*BRCA2* (13q12-q13), BRCA1	3.5- to 10-fold
Familial atypical multiple mole Melanoma syndrome (FAMMM)	*p16* (9p21)	20- to 34-fold
Peutz–Jeghers syndrome	*STK11/LKB1* (19p13)	75- to 132-fold
Ataxia-telangiectasia	*ATM* (11q22-23)	Undefined ↑risk
Familial adenomatous polyposis	*DP 2.5 (5q12-21)*	Undefined ↑risk
Familial pancreatic cancer	*BRCA2 (4q32-34)*	5- to 10-fold ↑ PC
Von Hippel-Lindau syndrome	*VHL (3p25)*	↑ Neuroendocrine tumors
Cystic fibrosis	*CFTR (7q31)*	↑ PC and GI cancers
Li-Fraumeni syndrome	*p53* (17p13.1)	↑ PC

Fanconi anemia	*FANC or FANCG* (3p22-26, 9p13, 9q22.3,16q24.3)	Slight ↑ PC
ABO blood group	*rs9543324* (13q22), *rs401681* (5p.33)	20- to 26-fold
Undefined familial PC	*PALB2*	Undefined ↑risk

Table 1: Inherited syndromes and susceptibility genes associated with an increased risk of pancreatic cancer

GI = Gastrointestinal. Reused from Yeo(17) *with permission, all rights reserved.*

2.2.3. HISTOLOGY AND PATHOLOGY

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent malignant tumor of the pancreas(12). The second most common is neuroendocrine tumors (NETs), followed by metastases from other primary tumors. Several distinct cystic precursor lesions exist with malignant potential, e.g., intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs). For the purpose of simplicity, the term PC will be used synonymously with PDAC in the rest of this thesis.

The current consensus is that malignant transformation in PDAC starts with acinar cells undergoing metaplasia and eventually forming premalignant precursor lesions called pancreatic intraepithelial neoplasia (PanIN)(18). PanIN lesions are graded from 1 to 3 in the order of severity based on the histological appearance. PanIN lesions are thought to represent different stages of gradually acquired mutations that eventually lead to uncontrolled cell division, invasive growth and metastasis, i.e., cancer. However, the linear progression from metaplasia to PanIN to PDAC has been disputed and may not represent the pathway of all pancreatic cancers(19).

2.2.4. DISEASE STAGES AND CLASSIFICATIONS

Several systems for staging and classification exist. The most commonly used staging guidelines are the American Joint Committee on Cancer (AJCC) 8th edition guidelines(20).

In the absence of metastatic lesions, the major determining factor for survival in PC patients is whether radical surgical resection can be performed(9). The limiting factor, in this regard, is the tumor's invasion of the surrounding vessel, as the resection/reconstruction of these vessels can be physically impossible, has major undesired consequences and/or has a high risk of nonradical surgery.

Several classification systems exist that focus on the resectability of the disease based on initial or subsequent imaging, e.g., the National Comprehensive Cancer Network (NCCN) guidelines(21) and the Karolinska Classification System (KCS)(22). These

guidelines offer guidance for when surgical resection is possible in nonmetastatic PC and thus are clinically relevant in the management of PC patients. Throughout this thesis, the term LAPC will be synonymous with the unresectable classification as defined by the NCCN(21) (Table 2).

Vessel	**Primary resectable**	**Borderline resectable**	**Unresectable**
PV/SMV	No contact Contact ≤ 180° without deformity	Contact > 180° Contact ≤ 180° with deformity/thrombus Contact with IVC	Unreconstructable obstruction Contact with proximal jejunal branch
CHA	No contact	Contact without extension to CA/HA-bifurcature (head tumors)	Contact with extension to CA/HA-bifurcature
CA	No contact	No contact (head tumors) Contact ≤ 180° (body + tail tumors)	Contact > 180° Any contact + contact with aorta
SMA	No contact	Contact ≤ 180°	Contact > 180° Contact with 1st jejunal branch Contact with aorta

Table 2: The NCCN classification system

PV = Portal vein, SMV = Superior mesenteric vein, IVC = Inferior vena cava, CHA = Common hepatic artery, CA = Celiac artery, HA = Hepatic artery, SMA = Superior mesenteric artery. Adapted from Tempero et al(21)

Several studies point out that a high level of or rapid increase in preoperative carbohydrate antigen 19-9 (CA-19-9) predicts adverse oncological results, e.g., inability to perform radical resection or lower overall survival (OS) after surgery(23–27). Based on these observations, some new guidelines have incorporated the CA-19-9 level as a biological factor for determining apparently resectable disease from borderline resectable PC (BRPC)(28).

2.2.5. TREATMENT

Treatment of PC is multimodal, meaning that several different aspects of treatment are applied in a coordinated effort. Although several important advances have been made, the progress has been largely incremental. In the earliest disease stages, upfront surgical resection with or without adjuvant chemotherapy is currently the preferred treatment. Several studies suggest that neoadjuvant chemotherapy may be beneficial, especially in BRPC(25,29). In LAPC, chemotherapy, with or without concurrent radiotherapy, may be useful to downsize the tumor to a resectable state. In nonresponding nonmetastatic tumors and in the presence of metastatic lesions, the only available treatment option is palliative chemotherapy, which may extend the life expectancy of the patients(5). Unfortunately, a large group of patients are not eligible for surgery or chemotherapy due to advanced age, comorbidity and/or low performance status (PS)(9). For these patients, the only treatment option is supportive care.

2.2.5.1 Surgery

Radical surgical resection is the only known treatment in PC with curative potential(16). The mainstay of pancreatic cancer surgery is pancreaticoduodenectomy (also known as the Whipple procedure), as this surgery can be performed in 56% of patients with primary resectable PCs, which are located in the head of the pancreas(30). The Whipple procedure involves the resection of the antrum of the stomach, duodenum, proximal jejunum, CBD, gallbladder and head of the pancreas (Figure 2). Other surgeries for primary resectable PC can be performed depending on the location of the tumor, e.g., distal pancreatectomy or total pancreatectomy.

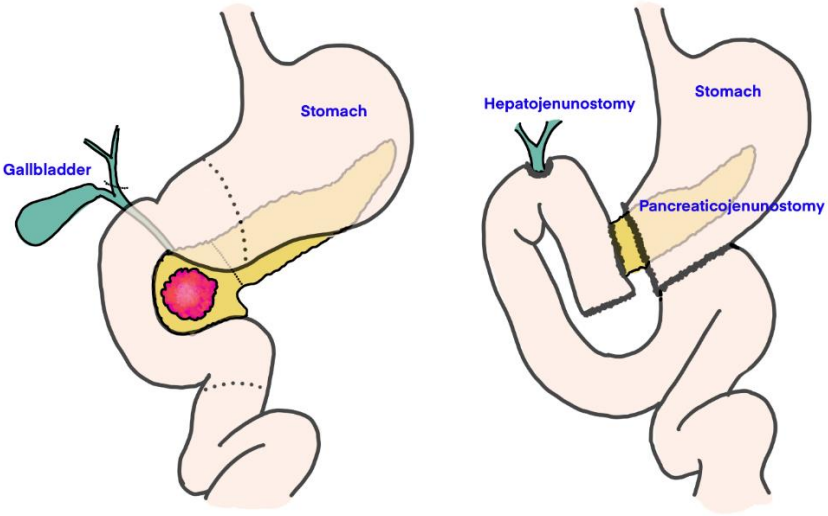


Figure 2: The Whipple procedure

Anatomy before (left) and after (right) a Whipple procedure.

In BRPC, conventional surgical techniques are combined with resection and, in most cases, the reconstruction of the blood vessels affected. The most common is venous resection, which is performed in approximately 25% of Whipple procedures, 5-35% of distal pancreatectomies and 50% of total pancreatectomies(31). Arterial resection of the CA (abbreviated CAR) may be performed if the GDA and CHA are not in contact with the tumor because this allows blood flow to the liver from the collateral arteries originating from the SMA. Several accounts of SMA resections have been reported in the research literature, but this procedure was correlated with high morbidity and mortality rates and low OS, thus making it inappropriate for clinical implementation at this point in time(32).

The radicality of the resection is stratified into three categories: R0, R1 and R2. R0 resections are microscopically radical, meaning that there is no microscopically visible tumor in the resection margin. The distinction between R0 and R1 is disputed, as some classification systems classify resections as R1 if there are tumor cells at the resection margin, while other systems define R1 resection if tumor cells are visible within one mm of the margin(33). The latter is the current consensus in Denmark. However, this distinction may not be sufficient because a substantial number of local

recurrences are observed even after R0 resections(12). R2 resections are defined by being macroscopically nonradical, i.e., with visible residual tumor after resection.

Pancreatic surgery for PDAC is associated with a high risk of complications(34), but the 30-day mortality rate after surgery is low in Denmark (approximately 1%)(35). The current 1-, 3- and 5-year survival rates after PDAC surgery in Denmark are 79%, 40% and 30%, respectively(35).

2.2.5.2 Chemotherapy

Chemotherapy is an important part of the multimodal treatment approach in PC. The advent of tolerable combination regimens, e.g., folinic acid (leucovorin) + fluorouracil + irinotecan + oxaliplatin (FOLFIRINOX) and gemcitabine + nab-paclitaxel, is likely the most important development in PC treatment during the past three decades(36). However, these combination regimens are associated with a high risk of toxicity and thus are appropriate only for patients with low comorbidity and good PS. While these combinations have had a large impact on survival, they are still associated with low response rates (approximately 25%)(37,38).

Chemotherapy can be instituted at different stages in the treatment, and the naming convention is based on the timing and purpose of the intervention. A schematic of the naming convention is portrayed in Table 3.

Term	**Timing**	**Purpose**
Adjuvant	After surgery	To target residual microscopic disease
Neoadjuvant	Before surgery in disease with high probability of radical resection (i.e., BRPC)	To increase the probability of radical surgery and eliminate microscopic disease
Downstaging/downsizing*	In the presence of unresectable disease without metastases	To downsize to resectable or borderline resectable disease
Palliative	Any time point when curative options are eliminated	To increase life expectancy and preserve quality of life

Table 3: Naming convention of chemotherapy in multimodal treatment

**Also termed induction chemotherapy*

For metastatic disease, palliative chemotherapy is the only treatment option. For LAPC, chemotherapy may be applied to downstage/downsize the disease to a resectable state(39). For BRPC, neoadjuvant chemotherapy may be beneficial prior to surgery, as studies have shown a longer OS compared to upfront surgery(25). However, this approach is disputed, as some studies have also shown that a smaller proportion of tumors are resectable after patients complete neoadjuvant chemotherapy(40). Nevertheless, neoadjuvant therapy may be useful in selecting which patients may benefit from surgery, as patients with distant progression on neoadjuvant treatment would likely experience rapid recurrence after resection(41,42). These results indicate that neoadjuvant chemotherapy may also have a place in the treatment of resectable PC. However, a recent meta-analysis failed to find any benefit of neoadjuvant chemotherapy in patients with resectable PC(29).

2.2.5.3 Radiotherapy

The role of radiotherapy in nonmetastatic PC treatment is highly disputed when applied in the neoadjuvant or downsizing/downstaging setting for primary resectable PC, BRPC or LAPC (42–45). Stereotactic body radiation therapy (SBRT) has been argued to provide better results than conventional intensity modulated radiation therapy (IMRT) because it delivers a higher radiation dose over fewer sessions(44,46). A recent meta-analysis found that LAPC patients treated with SBRT had a median OS of 14.1 months, while BRPC patients had a median OS of 17.5 months based on 19 published studies involving 800 patients in total(46). High-level evidence for SBRT in LAPC and BRPC treatment is currently not available.

2.3. ELECTROPORATION

Electroporation is a known, but not fully understood, process in which the stimulation of tissues or cells by pulsed electrical fields (PEFs) leads to transient or permanent pore formation in cellular membranes(47). This drastically increases the permeability of the electroporated membranes for practically all molecules. Several different therapeutic methods are based on electroporation as a core principle.

The earliest publication reporting the use of electroporation in living cells was published in 1982(48). In this experiment, the researchers showed that deoxyribonucleic acid (DNA) could be transferred into murine lyoma cells by subjecting the cells to an external electrical field, a process that has since been named gene electrotransfer (GET). The authors found that the optimization of the electrical field allowed DNA transfer without damaging the ablated cells. The work of several other research groups throughout the 1980s and 1990s refined the technique, leading to the optimization of electrical pulse delivery to provide transient pore formation without inducing cell death, i.e., reversible electroporation (RE). The first clinical trial using therapeutic electroporation was published in 1993 by Belehradek et al.(49). In

this study, systemic chemotherapy was combined with local ablation using RE (termed electrochemotherapy (ECT)), and the feasibility of ECT in cancer treatment was demonstrated. The field has been rapidly evolving ever since, and ECT is now considered the standard of care for some specific skin tumors(50).

ECT is an enticing treatment entity because it allows for enhancement of the cytotoxic effects of chemotherapy in the ablated area without increasing systemic side effects. This ability is based on two known mechanisms. The first and most important mechanism is the intracellular capture of chemotherapy agents due to the transient increase in permeability. The second mechanism is termed the vascular lock effect. RE-treated cells tend to swell, which hinders perfusion and thus the clearance of chemotherapy agents in the treated area. The increase in the effectiveness of several different chemotherapeutic agents has been tested in vitro. The most commonly used agents are bleomycin and cisplatin because they have shown 150- to 5000-fold and 3- to 12-fold increases in cytotoxicity in different cancer cell lines, respectively(51). Since the inception of the use of ECT in superficial cancers, technological developments have continued, leading to the creation of several advanced needle electrode designs allowing RE ablations in nearly all tissues and tumor sites(52).

Chemotherapy during ECT can be administered via two different routes. Intravenous (i.v.) injection is preferred for larger tumors, whereas for smaller tumors, chemotherapy may be injected directly into the tumors (intratumoral (i.t.) injection). A European Standard Operating Procedure for ECT for cutaneous tumors has been developed and is widely adopted in the research literature(53).

Another important and interesting combination with electroporation is i.t. calcium injection (termed calcium electroporation (CaEP)), which has been shown to be feasible in PC(54). CaEP works by depleting adenosine triphosphate (ATP) in treated cells and may be selective toward cancer cells(55). Additionally, CaEP is completely nontoxic to the rest of the organism, which makes it a highly interesting and clinically relevant enhancing agent.

The earliest studies describing what is now termed IRE go back to the 1950s, but the findings were largely ignored in medicine until 2005(56,57). IRE has a similar mechanism of action as RE but uses higher electrical field strengths and more pulses to destroy cells in the ablated area without the need for adjuvant therapies. IRE causes unrecoverable pore formation in the cellular membranes, leading to a loss of homeostasis and, in turn, cell death. IRE ablation has theoretical advantages over conventional thermal ablation modalities, e.g., radiofrequency ablation (RFA) and microwave ablation (MWA). Most importantly, IRE causes cellular death without destroying tissue scaffolding, which forms physical anatomical barriers, e.g., vessel walls. Consequently, IRE can be performed in areas with critical structures such as major blood vessels without affecting the integrity of these structures. Moreover,

because of its nonthermal mechanism of action, IRE is not affected by the heat-sink effect, which can affect the technical efficacy of thermal ablations(58,59).

2.4. IRREVERSIBLE ELECTROPORATION IN PANCREATIC CANCER

Ablation with IRE has been proposed as a new consolidative treatment option in patients with LAPC(60). This proposal is based on the theoretical benefits of IRE over other ablative modalities, especially because of the favorable effect of IRE on vessels in the treatment zone, which is the major limiting factor for achieving radical resection (as described in section 2.2.5.1). IRE treatment may be conducted on its own or in combination with other simultaneous therapies to achieve a synergistic effect (such as ECT or CaEP). When used without medical agents, IRE can be applied in situ or in conjunction with surgical resection. The latter has been termed margin accentuation, as IRE treatment is performed only in the field where there is a high risk of nonradical resection, i.e., along the SMA. In situ IRE aims to completely ablate the tumor to eradicate most or all cancer cells to achieve local control or to downstage/downsize to a resectable stage. In situ IRE can be performed during open surgery(61–63) or percutaneously with image guidance(1,64–68).

A novel development of IRE is high-frequency IRE (H-FIRE). H-FIRE uses shorter pulse lengths than conventional IRE and switches the positive and negative poles of the needles between each pulse delivery. This method has been shown to significantly reduce muscle contraction during energy delivery and thus may require less muscle relaxation and cause less pain than conventional IRE(69,70). However, only a few clinical trials of H-FIRE have been conducted to date(71).

The application of IRE in LAPC is still considered a topic of research by national guidelines, e.g., the National Institute for Health and Care Excellence (NICE)(72). From a theoretical standpoint, IRE may be beneficial because 30% of patients with pancreatic cancer die as a result of local tumor infiltration without evidence of metastatic disease in autopsy(73). Several studies of IRE in LAPC have been conducted to date(61–68,74–78). The results of these will be discussed in Chapter 6.

CHAPTER 3. OBJECTIVES

The overall aim of this thesis is to evaluate the current role of IRE in LAPC treatment, to identify important knowledge gaps and to explore the future perspectives of IRE in PC. Four studies were conducted as part of this effort. The objectives of the individual studies are listed below.

3.1. STUDY 1

- To examine the safety and feasibility of IRE in patients with LAPC.

3.2. STUDY 2

- To examine the use of and evidence for imaging response evaluation in LAPC after local ablative interventions.
- To make recommendations on imaging response evaluation in future trials.

3.3. STUDY 3

- To examine the correlation between imaging response evaluation and survival in PC patients treated with IRE.

3.4. STUDY 4

- To examine whether a transient zone of electroporation exists in conjunction with the IRE zone after IRE.
- To examine the distribution of bleomycin after IRE with concurrent i.v. bleomycin.

CHAPTER 4. MATERIALS AND METHODS

4.1. STUDY 1

4.1.1. STUDY DESIGN

An observational cohort study was designed to prospectively follow patients treated with IRE at Aalborg University Hospital from October 2013 to March 2018(1).

4.1.2. PATIENT SELECTION

Referrals for the study were open from all four Danish PC centers. Upon referral, patients were first screened by the pancreatic multidisciplinary team (MDT) conference at Aalborg University Hospital(1). To be included, patients had to have LAPC according to the NCCN criteria. All candidates were then scanned by ^{18}F-FDG PET/CT with triple-phase intravenous contrast to ensure proper staging. Patients with borderline resectable disease or suspected metastatic lesions were referred to a second hospital for surgery or to the oncological department for chemotherapy. Patients were required to receive chemotherapy before entering the trial unless this was contraindicated by comorbidity, etc. The choice of chemotherapy was not standardized and differed between the referring institutions. Patients were excluded if they were under 18 years of age; had tumors larger than five cm in any diameter; were pregnant; had severe chronic heart disease, cardiac arrythmias, cardiac pacemakers or implanted cardiac defibrillators, or an Eastern Cooperative Oncology Group (ECOG) performance status (PS) > 2; or were unable to give informed consent.

4.1.3. ABLATIVE PROCEDURE

The selected patients were treated with in situ IRE using the following methods. Patients were treated under general anesthesia with deep neuromuscular blockade (train of four (TOF) = 0)(1). External pacing electrodes were placed to ensure rapid defibrillation or pacing in case of cardiac arrhythmias as a safety precaution. Patients were given intravenous beta-blockers (metoprolol) prior to electroporation to alleviate procedure-related hypertension. Ablations were performed using the NanoKnife system (Angiodynamics, Queensbury, NY, USA). Two to six needle electrodes were placed around or inside the tumor tissue with a maximum distance of 2.5 cm by an expert radiologist using ultrasound (US) guidance. The specific number and arrangement of needles differed between patients and was chosen based on the likelihood of achieving complete ablation. In a few patients with large tumors, six needles were not enough to cover the entire tumor in the anterior-posterior direction. In these patients, rearrangements of needles were performed. The exposed active tips

of the needles were between one and two centimeters and were chosen on a case-by-case basis. Needle tip alignment and uniform depth were ensured prior to treatment. The electrical pulses were synchronized with the refractory period of the electrocardiogram using a device. Pulse length was fixed at 90 ms. To perform successful ablation, the goal was to administer at least 90 pulses with each needle pair with a current of >30 amperes. This goal was achieved by first administering 20 test pulses with each pair using an electrical field strength of 1500 V/cm. In pairs that did not reach the threshold, the field strength was turned up gradually and tested individually until the goal was reached. When all pairs had achieved the desired current, 70 additional pulses were administered. In tumors with a large diameter on the sagittal axis, needles were pulled back one to two centimeters, and the treatment regimen was repeated. Finally, an US scan was performed to look for signs of bleeding. After the procedure was concluded, patients were observed for complications in the surgical ward for at least 24 hours.

4.1.4. ENDPOINTS

The endpoints included several aspects of safety, i.e., the 90-day complication rate and severity (Clavien–Dindo (CD) grade(79,80)), 30-day mortality, length of hospital stay and postoperative pain severity(1). Feasibility was assessed by the treating physician and defined as the ability to place the needle electrodes around or within the tumor with US guidance and to administer 90 pulses with each needle electrode pair with at least 30 amperes. All endpoints regarding safety and feasibility were reported per ablation procedure performed, as several patients were treated more than once. Finally, we examined whether OS was affected by the baseline tumor size and whether repeated IRE treatment would lead to increased morbidity.

4.1.5. FOLLOW UP

Patients in the cohort were followed until death or until the study was concluded (September 2018)(1). Patients were followed using ^{18}F-FDG PET/CT with triple-phase intravenous contrast every three months for up to two years. Oncological treatment, surgical explorations/resections and survival time were recorded during follow-up.

4.1.6. STATISTICS

The primary outcomes were summarized by the absolute number and rate or by median and range, as these were markedly distribution free(1). Survival time was illustrated by Kaplan–Meier plots, and differences were tested using a log-rank test. Differences in proportions were tested using Fischer's exact test. A p value $< .05$ was considered significant. Plots and statistics were created using Stata version 15.1 (StataCorp LLC, College Station, TX, USA).

4.1.7. ETHICS

The study protocol was reported to the North Denmark Region Committee on Health Research Ethics before initiation and was found to be exempt due to Danish law regarding CE-marked device interventions at the time(1). Informed consent was obtained from all patients prior to any study-specific interventions. The study protocol was made available at (http://www.clinicaltrials.gov/, NCT02079623).

4.2. STUDY 2

4.2.1. STUDY DESIGN

To examine the use of and evidence for imaging response evaluation in ablation therapies for LAPC, an expedited systematic review was performed, largely adhering to the Preferred Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines(2,81,82). An expedited approach places less emphasis on certain aspects of the review process and was chosen in this case, as this was more aligned with the exploratory objectives of this review. The minimized aspects, in this case, were the assessment of bias, the publication of a review synopsis in public databases, meta-analysis and literature search in multiple databases.

4.2.2. LITERATURE SEARCH STRATEGY

The search strategy was planned by creating search categories using the PICOS concept, by which the following categories were defined: population, intervention, comparison, outcome and study type(2). Based on this, and with the aid of a dedicated research librarian, a search was performed in the PubMed database on April 11th, 2019. The final search string was as follows:

> (((((("Pancreatic Neoplasms"[Mesh] OR ((pancreatic[TW] OR pancreas[TW]) AND (cancer* [TW] OR neoplasm*[TW] OR adenoma*[TW] OR carcinoma*[TW]))))))) AND ((((((((((("Electroporation"[Mesh]) OR "Radiofrequency Ablation"[Mesh]) OR "High-Intensity Focused Ultrasound Ablation"[Mesh]) OR "Cryosurgery"[Mesh]) OR radiofrequency ablation*[TW]) OR microwave ablation*[TW]) OR high-intensity focused ultrasound ablation*[TW]) OR cryosurger*[TW]) OR cryoablation*[TW]) OR electroporation*[TW]) OR electropermeabilisation*[TW]) OR electropermeabilization*[TW]) OR electrochemotherap*[TW])

4.2.3. SELECTION PROCESS AND DATA EXTRACTION

The selection of articles to be included was carried out in two rounds using the online software solution Covidence (Veritas Health Innovation, Melbourne, Australia)(2,83). Articles were screened for inclusion by reading titles and abstracts. All primary studies regarding in situ ablation of LAPC using nonchemical- and nonradiation-based approaches and with a primary or secondary imaging endpoint were included in the first round. Because of differences in reporting, stage III was considered synonymous with LAPC in this review. In the second round, the full texts of all articles were read.

An article was excluded from the review if it included fewer than five patients; if it was impossible to differentiate the results from different interventions/disease stages/histological origins; if the full text could not be acquired within one month from inquiry; if the publishing language was not English; if the article was an obvious duplicate (same outcomes and number of patients from the same authors); if the study combined different procedures, e.g., surgical resection with margin accentuation; and if the imaging assessment was not related to efficacy evaluation, e.g., imaging assessments of vessel patency after ablation.

The screening and exclusion of articles were performed by a single author. Quality control was carried out by a second author by screening 10% of the excluded articles. Any disputes were settled by consensus.

Finally, data extraction was performed by a single author, and 100% of the data were checked by one of three coauthors. The outcomes extracted were the author, year of publication, country (first author), number of LAPC patients treated, ablation modality, imaging modality and assessment method (e.g., functional magnetic resonance imaging parameters), response evaluation method (comparative), response evaluation target (patient-level (termed systemic) or lesion-level (termed local), timing of the evaluated outcome (< 3 months after ablation, termed short-term or ≥ 3 months, termed long-term) and baseline scan for comparison. Furthermore, the methods used were characterized as standardized or nonstandardized. Standardized methods were defined as criteria published by international groups, e.g., Response Evaluation Criteria in Solid Tumors (RECIST).

4.3. STUDY 3

4.3.1. STUDY DESIGN

Based on the results of Study 2, a lack of evidence in this area was identified. Therefore, a study examining the correlation between imaging outcomes and survival

was performed using data from Study 1 that were not previously published(3). Data from all standardized PET/CT scans were gathered from patients in the cohort along with baseline data and survival information. Patients were scanned every three months during follow-up for up to two years or the withdrawal of consent. Follow-up was stopped in March 2020. Contrary to Study 1, the cohort for this study included not only patients with LAPC but also patients treated with IRE for resectable tumors (e.g., patients with severe medical comorbidities in whom surgery was contraindicated) or isolated local recurrence after resection.

4.3.2. IMAGING PROTOCOL

^{18}F-FDG PET/CT was performed in all patients in accordance with institutional practices(3). The image acquisition protocol was planned according to the European Association of Nuclear Medicine guidelines(84). The ^{18}F-FDG doses ranged from 172 to 400 Mbq (mean 347 MBq). Images were obtained from the base of the skull to the upper thigh. CT images of the abdomen were obtained in three phases (prior to contrast injection, in the arterial phase and in the portal venous phase) using an intravenous iodinate contrast agent (Iomeron® 400 mg iodine/ml). All images were obtained using either a VCT Discovery True 64 PET/CT system (GE Healthcare, North Richland Hills, TX, USA) or a Siemens Biograph mCT Flow 64 PET/CT system (Siemens Healthineers, Erlangen, Germany).

4.3.3. IMAGING ANALYSIS AND ENDPOINTS

Two separate imaging experts (one radiologist and one nuclear medicine specialist) reviewed all images(3). Differences in interpretations were solved by consensus. The outcomes included 1) the largest tumor diameter of the pancreatic lesion on CT, 2) the maximum standard uptake value (SUV_{max}) of the pancreatic lesion, 3) the metabolic tumor volume (MTV) of the pancreatic lesion, 4) the total lesion glycolysis (TLG) of the pancreatic lesion, 5) the number of suspected metastases on CT and 6) the number of PET-avid suspected metastases. The SUV, MTV and TLG were automatically calculated in a reviewer-defined volume of interest (VOI) using the software SyngoVia VB40 (Siemens Healthineers, Erlangen, Germany). The MTV was defined based on voxels with an SUV > 2.5. The TLG was calculated by multiplying the MTV by the mean SUV (SUV_{mean}).

The outcomes were compared between scans for each patient. Follow-up scans were compared to either the baseline scan (prior to IRE ablation) or the first subsequent scan, as recommended by the Society of Interventional Radiologists (SIR)(85). Scans performed after 6 months were censored, as only a few patients had received scans after this time point and because several patients were either re-treated with IRE or went on to undergo surgical resection after this timepoint. Lesion-level and patient-level outcomes were reported separately to examine the two different approaches being utilized in this research field(2).

4.3.4. SURVIVAL ENDPOINTS

Treatment dates and dates of death were acquired through patient charts(3). The treatment date for the first IRE treatment was considered the entry date for the time-to-event analysis, and the date of death was considered the exit date. Patients still alive on March 21st, 2020, were censored from this timepoint.

4.3.5. STATISTICS

Comparative lesion-level outcomes were either dichotomized and defined as progressive disease (PD) or non-PD or analyzed as continuous variables to explore correlations without losing statistical power(3). The cutoff for the CT outcome was a ≥ 20% increase, based on RECIST 1.1(86). The threshold for PD for PET outcomes was a ≥ 25% increase based on the cutoff used in the European Organization for the Research and Treatment of Cancer (EORTC) PET response criteria(87).

Patient-level outcomes were created in a similar dichotomized fashion in accordance with RECIST 1.1(86) for CT parameters or the EORTC PET response criteria(87) for PET parameters. Patient-level PD according to RECIST 1.1 was defined as a ≥ 20% increase in the ablated tumor diameter or an increasing number of suspected metastases. Patient-level PD according to the EORTC was defined as a ≥ 25% increase in the SUV_{max} or any increase in the number of PET-avid suspected metastases.

The imaging endpoints, i.e., response category according to the different definitions, were then correlated with survival time using Poisson regression with the Huber–White Sandwich estimator(88). In the first analysis, the dichotomized values were analyzed, as these were clinically relevant. Due to the low sample size, additional analyses at the lesion level were performed with outcomes as continuous variables as an exploratory analysis with more statistical power. Multivariate analysis was not performed, as the authors thought that the sample size was too small. All statistical analyses were performed using Stata version 16.1 (StataCorp LLC, College Station, TX, USA) by a dedicated biostatistician.

4.4. STUDY 4

4.4.1. STUDY DESIGN

To examine and characterize the proposed RE zone and the feasibility of synchronous chemotherapy/IRE treatment, an animal experimental study was designed (Appendix (App.) 4). The study was performed in five healthy farm pigs. All animals were subjected to IRE ablation of the liver and pancreas with concurrent intravenous injection of a magnetic resonance imaging (MRI) contrast agent and bleomycin.

Afterward, animals were scanned in an MRI scanner, and finally, the ablated lesions were excised and frozen for later bleomycin quantification. To differentiate the RE zone from edema/inflammation, two control ablations were performed. Due to size limitations, control ablations could be performed in the liver but not in the pancreas. The first control ablation was performed using IRE settings but was carried out two hours prior to contrast/bleomycin injection and thus represented a negative control. The second control ablation was performed shortly after the contrast/bleomycin injection using RE settings, typically used for ECT treatment, representing a positive control. The timeline of the experiment is available in Figure 3 (App. 4).

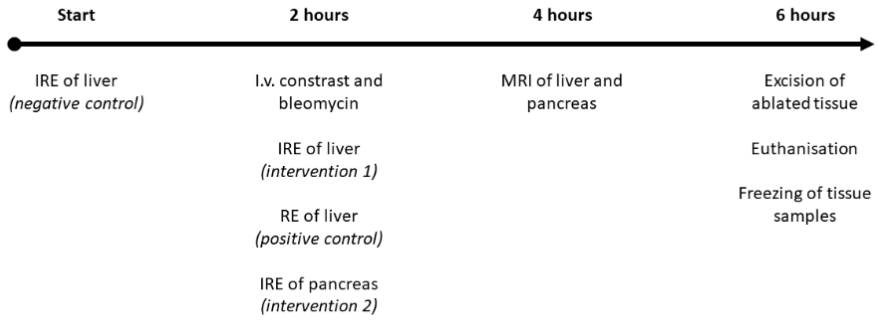


Figure 3: Study 4 timeline

4.4.2. ELECTROPORATION PROTOCOL

Two different setting were used. IRE ablation settings were adapted from guidelines for IRE ablations in pancreatic cancer(89), while the RE settings were modified from the updated European Standard Operating Procedure for ECT (ESOPE) in cutaneous tumors(53) (App. 4). For the latter, a pulse frequency that was slower than that recommended by the guidelines was chosen because of limitations in the EP system used. The needle electrode distance, tip exposure and number of probes were standardized to increase comparability. All EP ablations were executed using the NanoKnife system (Angiodynamics, Queensbury, NY, USA). The exact EP parameters are presented in Appendix 4.

4.4.3. MAGNETIC RESONANCE IMAGING

MRI was performed without contrast enhancement using a clinical magnetic resonance scanner (Philips – Ingenia 3.0T) (App. 4). Respiratory movements of the

animals were minimized by using apneic oxygenation(90). In the first animal, several imaging techniques were performed to find the best-suited method for separating the normal tissue, contrast-enhancing tissue and hypointense tissue of the liver. During this stage, it was not possible to clearly differentiate the pancreatic lesions from normal pancreatic tissue in a measurable way. Therefore, the final imaging protocol and analysis was focused on liver ablation. The final imaging protocol was T1-weighted fast-field-echo (Philips FFE) images acquired in 3D mode with an isometric 1.5 mm^3 voxel reconstruction (App. 4).

4.4.4. TISSUE SAMPLING AND PRESERVATION

Upon the completion of MRI, animals were returned to the animal laboratory (App. 4). Large tissue samples from the four ablation sites were excised. All resected tissue samples were cut longitudinal to the needle placement and divided into approximately 20 x 60 x 5 mm samples. Tissue samples were then immediately frozen at -80 °C for later analysis (App. 4).

4.4.5. IMAGING ANALYSIS

The attained images were quantitatively analyzed using the free open-source application 3D slicer (version 4.11)(91,92) (App. 4). The mean liver parenchymal intensity for each animal was found by creating larger segmentations of the nonablated areas of the livers in three planes. Afterward, the individual ablative lesions were identified and segmented separately. The individual lesions were first segmented in total by using the surface cut (smooth model) tool by visual differences in contrast. Then, the individual lesion segmentations were separated into two (the hyper- and hypointense volumes) using the mean liver intensity as the threshold for separation. Quantitative values, including lesion volume, mean lesion intensity and lesion intensity standard deviation, were then extracted for statistical analysis (App. 4).

4.4.6. BLEOMYCIN DETECTION AND QUANTIFICATION

Bleomycin detection and quantification were performed using either mass spectrometry-based imaging or liquid chromatography coupled with mass spectrometry (App. 4). The full description of the procedure is available in App. 4.

4.4.7. STATISTICS

All statistical analyses were performed using Stata version 16.1 (StataCorp LLC, College Station, TX, USA) (App. 4). Parametric data were reported as the means and standard deviations (SD). Differences in nonnormally distributed data were tested using the Kruskal–Wallis test. A p value $\leq .05$ was considered statistically significant (App. 4).

4.4.8. ANIMAL ETHICS

The experimental animals were anesthetized prior to the study intervention and remained under anesthesia until they were euthanized (App. 4). The protocol was approved by the Danish National Animal Experimentation Council on October 3rd, 2018. The study design adhered to the 3R principles. All personnel had the required animal research licensing. The study procedures and associated animal care were overseen by a licensed veterinarian (App. 4).

CHAPTER 5. SUMMARY OF RESULTS

5.1. STUDY 1

5.1.1. STUDY POPULATION

- Thirty-three patients with LAPC underwent a total of 40 IRE ablations during the study period(1).
- The median time from diagnosis to the first IRE ablation was 5.3 months (range 0.7-14)(1).
- Baseline demographics are displayed in the published article(1).

5.1.2. FEASIBILITY

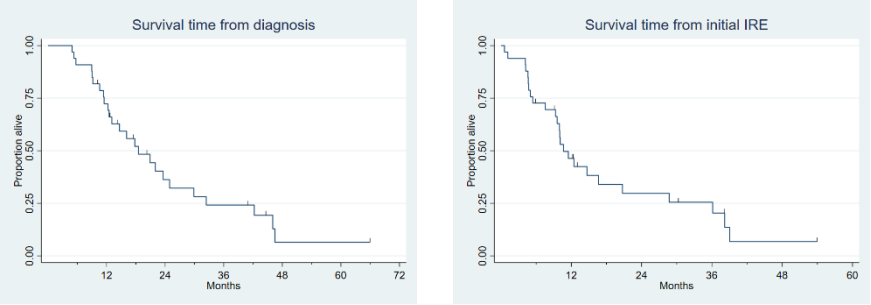
- All 40 ablations were successful based on the stated criterion(1).

5.1.3. SAFETY

- Hospitalization lasted a median of one day (range 1-13)(1).
- The median perceived perioperative pain on the visual analog scale (VAS) was zero before IRE ablation and was four, one, one and one, two hours, eight hours, one day and four weeks after IRE ablation, respectively(1).
- A major adverse event (CD grade $\geq$ 3) occurred within 90 days in 20% of ablations (n = 8)(1).
- The 30-day mortality rate was 5% (n = 2)(1).
- The rate of severe events (CD grade $\geq$ 3) tended to be higher in relation to retreatment (43% vs. 15%, p=.13)(1).
- Larger tumors (largest diameter $\geq$ 3.5 cm) had a trend toward higher rates of overall complications (67% vs. 41%, p=.13) and severe complications (28% vs. 14%, p=.43)(1).

5.1.4. ONCOLOGICAL RESULTS

- The median OS for the cohort was 10.7 months and 18.5 months from the intervention and the diagnosis, respectively (Figure 4)(1).
- The tumors of three patients were downsized, and these patients went on to undergo surgical resection (two R0 and one R1)(1).
- Patients with larger tumors (largest diameter $\geq$ 3.5 cm) had a significant decrease in median OS compared to patients with smaller baseline tumors (7.6 months vs. 16.7 months, p=.02)(1)



(a) **(b)**

Figure 4: Plots of survival from (a) diagnosis and (b) the initial IRE treatment

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5.2. STUDY 2

5.2.1. STUDY POPULATION

- Thirty-four studies were included from an initial total of 713 studies identified by the search strategy (Figure 5)(2)
- The interventions in the included studies, in the order of frequency, were IRE, RFA, high-intensity focus US (HIFU), MWA, ECT and cryothermal probe ablation (CTP)(2).

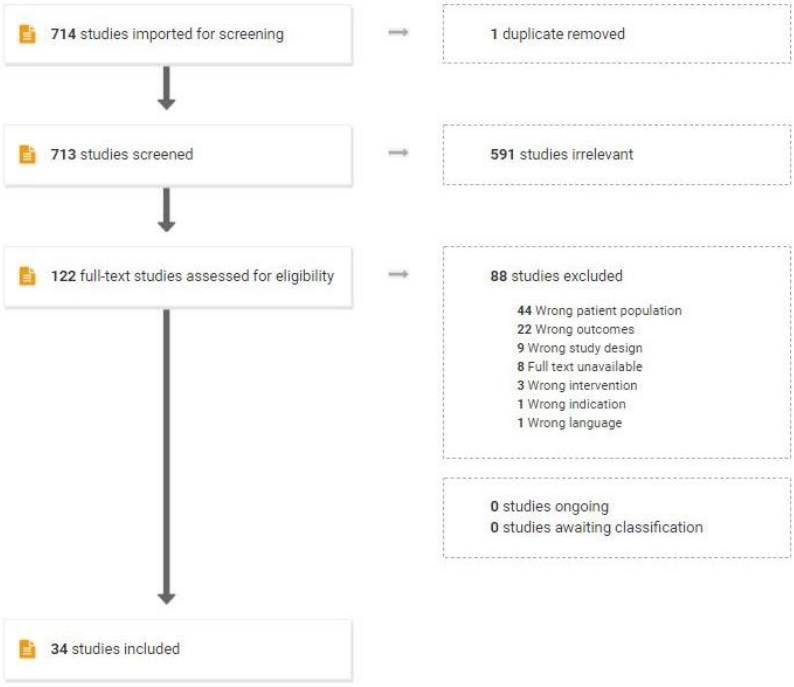

Figure 5: CONSORT diagram of the study selection strategy

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5.2.2. IMAGING ASSESSMENT METHODS

- CT scans were used in the response evaluation strategy in all but one study. One study used only MRI(2,93).
- In thirteen studies, the use of more than one imaging modality was reported(2,65,77,94–104). However, in nine studies, different imaging modalities were interchangeably used across patients or time(2,65,77,94–97,102–104).
- Standardized criteria, i.e., RECIST(86), PERCIST(105); Choi response criteria (Choi)(106); and modified RECIST (mRECIST)(107) for response evaluation were used in 14 studies(2,67,74,96,97,99–101,103,104,108–112).
- In fourteen studies, only self-determined predefined criteria, simple size comparisons or qualitative descriptions were reported(2,63,65,68,93,95,98,113–119).
- In six studies, the methods used for imaging response evaluation were not reported(2,61,75,94,102,120,121).

5.2.3. TARGET FOR RESPONSE EVALUATION

- In most studies (n = 27), the target for comparison was reported (patient-level(74,75,94,97,121), lesion-level(93,95,96,98–101,108–110,113–115,117–119) or both(61,65,68,77,116))(2).

5.2.4. COMPARISON SCAN FOR RESPONSE EVALUATION

- In sixteen studies, follow-up scans were compared to the preablative scan(2,67,93,95,98–102,109–111,113–115,117,118).
- In two studies, the first postablative scan was used for comparison with long-term follow-up scans, as recommended by the SIR(2,68,77,85).
- In a substantial number of studies (n = 16), it could not be determined which scan was used for comparison(2,61,63,65,74,75,94,96,97,103,104,108,112,116,119–121).

5.2.5. TIMING OF RESPONSE EVALUATION

- In a total of fifteen studies, both short- (< 3 months) and long-term (≥ 3 months) outcomes(2,67,74,75,77,93,96–98,103,104,109,112,114,116,119) were reported, while the rest reported either one or the other.

5.2.6. CORRELATION BETWEEN IMAGING OUTCOMES AND SURVIVAL

- In one out of the 34 studies, the correlation between imaging outcomes and survival was tested, and the results showed that early disease progression based on self-defined criteria was correlated with survival (p=.003)(2,68).

5.3. STUDY 3

5.3.1. STUDY POPULATION

- Forty-one patients with LAPC (n = 33), isolated local recurrence (ILR) (n = 4) or inoperable due to severe comorbidity PC (MIPC) (n = 4) were included in the study cohort(3).
- All patients were scanned by PET/CT at baseline. Thirty-five and 22 patients were scanned during follow-up at three months and six months, respectively(3).

5.3.2. PATIENT-LEVEL ANALYSIS

- PD according to RECIST was significantly correlated with a higher mortality rate (MR) during all three time-intervals (Table 4)(3).

- PD according to the EORTC was significantly correlated with MR only in the long time interval (baseline to six months) and completely failed to differentiate patients in the postablative interval (three to six months)(3).

PD criteria	Comparison	Time-interval	MRR	Lower 95% CI	Upper 95% CI
RECIST 1.1	Baseline	0–3 months	**2.646**	1.253	5.587
	Baseline	0–6 months	**2.546**	1.033	6.273
	Post-ablative	3–6 months	**7.075**	2.944	17.000
EORTC	Baseline	0–3 months	1.420	0.537	3.757
	Baseline	0–6 months	**1.829**	1.108	3.020
	Post-ablative	3–6 months	*	*	*

Table 4: Patient-level outcome analysis

*Significant results in bold. * Results not shown because all patients were characterized as progressors. MRR = Mortality rate ratio. CI = Confidence interval. Reused from Flak et al*(3) *with permission, all rights reserved.*

5.3.3. LESION-LEVEL ANALYSIS

- None of the dichotomized outcomes in the lesion-level analysis were significantly correlated with the MR(3).
- The MTV and TLG approached statistically significant results in all time intervals(3).
- Analysis with outcomes as continuous variables revealed that the tumor size increase was significantly correlated with the MR in the long time interval and in the postablative interval and approached significance during the early interval (baseline to three months)(3).
- Increases in the MTV and TLG were both significantly correlated with an increased MR in the long and postablative intervals but were not correlated with MR (MRR $\leq$ 1) in the first interval(3).
- The SUV_{max} did not correlate with the MR in any of the time intervals(3).

5.4. STUDY 4

5.4.1. IMAGING RESULTS

5.4.1.1 Qualitative description

- The liver ablation sites were visible on T1-weighted FFE images, whereas the pancreatic ablation sites were not easily distinguishable from normal pancreatic tissue (App. 4).
- The IRE lesions with concurrent contrast (I1) appeared as a hypo-/isointense inner part surrounded by a hyperintense penumbra (App. 4).
- In some animals, hyperintensity was present near the needle path, and near the vessels that were included in the ablation zone (App. 4).
- The IRE control lesions (C1) resembled the I1 lesions but without the hyperintense penumbra (App. 4).
- The RE control lesions with concurrent contrast (C2) were smaller than the I1 and C1 lesions but resembled the intensity patterns seen in the I1 lesions (App. 4).

5.4.1.2 Imaging segmentation

- The mean total volumes of the I1 lesions were larger than those of the C1 lesions (37.2 vs. 19.6 cm^2, p=.03) (App. 4).
- The difference in total volume was almost completely composed of the hyperintense volume (20.2 vs. 2.7 cm^2, p=.009) (App. 4).
- The mean difference in intensity between the hyperintense volumes and the mean liver intensity were 96.0, 31.2 and 83.9 for the I1, C1 and C2 lesions, respectively (p=.03) (App. 4).

5.4.2. MASS SPECTROMETRY RESULTS

- Bleomycin could not be detected in the tissue samples (App. 4).
- Additional quality control was performed, and it was found that bleomycin was present in the blood at the time of ablation (App. 4)

CHAPTER 6. DISCUSSION

PC is a devastating illness with a large impact on afflicted patients' lives. Curative treatment options are few and are associated with a major risk of complications even in high-volume centers(122,123). Importantly, a large group of patients are not eligible for any treatment, which leads to a premature loss of life and devastation for patients and their families. While some incremental advances have been made, mainly in the form of more effective combination chemotherapy regimens, palliative treatment options will fail due to resistance or intolerance for all patients at some point. Thus, innovations in surgical and medical treatments are highly desirable.

The hallmarks of evidence-based medicine are that any new treatment must be proven to be safe and effective. While it is clear what constitutes an efficacious treatment and how to test treatment efficacy (i.e., randomized controlled trials (RCTs)), it is much less clear what constitutes a safe treatment and how to interpret whether a given effect size warrants a risk of complications. In either case, informed and joint decision-making is needed, especially if a treatment option is not yet well established, i.e., backed by clear, validated and irrefutable evidence.

In the following chapter, the results of the studies included in this thesis will be discussed, and the results will be put into the context of the established knowledge on the subject. The research field is rapidly evolving, with several new studies being published each month. A summary of the state of the art will be presented, and future areas of research will be identified.

6.1. FEASIBILITY

The feasibility of IRE in LAPC treatment has been clearly demonstrated by multiple publications, including Study 1 of this thesis(1,124). Furthermore, because IRE can be performed in multiple ways, i.e., percutaneously, during laparotomy and as margin accentuation, there are multiple ways that IRE can be implemented clinically. The learning curve of open in situ IRE in LAPC has been demonstrated in a single publication, which showed that physicians were able to perform the procedure in more advanced patients after performing the procedure in only five simple cases(125).

6.2. PATIENT ELIGIBILITY

Most patients are eligible to undergo IRE, as absolute contraindications are few. Nevertheless, because the prevalence of comorbidities increases with age, it is

important to exclude patients who have a high a priori risk of adverse outcomes. When a new therapy is introduced, contraindications can be derived only from theory. However, as more studies regarding the potential contraindications for pancreatic IRE are published, the acquired knowledge is slowly proceeding beyond theory and into practice.

Cardiac arrythmias have been reported after IRE(126), and thus, preventative measures are taken to minimize this risk, mainly the synchronization of pulse delivery with the ECG and preoperative placement of pacing pads for quick cardioversion or pacing in the case of arrythmias(127). Implanted cardiac pacemakers or analogous devices have thus far been seen as a contraindications for IRE, but a new study suggests that EP treatments may be both safe and effective in this patient group(128). One important limiting factor is persistent atrial fibrillation, as electrical pulses cannot be consistently delivered in the refractory period of the ECG as they may induce cardiac arrythmias. However, it is likely that innovations such as H-FIRE, where ECG synchronization is not required, may alleviate this limitation in the future(69,70).

Patients with a history of seizures are not eligible to undergo IRE as a precautionary principle.

Patients with poorly treated or uncontrollable hypertension are not eligible for IRE at this time due to a transient increase in blood pressure during energy delivery. Steps can be taken to lessen the increase in blood pressure, e.g., beta-blockade and deep anesthesia, but more evidence is needed to ensure whether this is enough to reduce the risk of brain hemorrhage(129).

Some researchers have argued that it is unsafe to perform IRE in the presence of self-expanding metal stents (SEMSs)(130), but this has since been disputed(131,132) and investigated further(133,134). One study performed IRE in a gel-based model and found that IRE led to increased heating near the needle electrodes in the presence of SEMSs, but no heating of the SEMSs was observed(133). This was later tested in an animal model. In this experiment, researchers found that white coagulation, as is common with thermal ablation, occurred only near the needle electrode tips, but interestingly, a small rim of vital tissue remained around the SEMSs, suggesting that SEMSs may affect technical efficacy. Similarly, another study examined the electrical field distribution around SEMSs in a castor-oil-based model and in computer simulations and found that the electrical field was disturbed by the presence of SEMSs, potentially leading to incomplete ablation(134). Interestingly, IRE ablation has been demonstrated as a method for recanalizing an occluded biliary stent via a proprietary experimental IRE catheter in an animal model(135). In conclusion, it is not yet clear whether IRE in the presence of SEMSs is safe, but it seems likely that the presence of SEMSs will not be a contraindication in the future. Importantly, there may be a distinction between the safety profiles of fully coated and uncoated SEMSs.

Another factor limiting the use of IRE in LAPC may be large tumor size, as was demonstrated in Study 1. In particular, if the tumor invades the duodenum, this theoretically may cause perforation when the treated tissue goes through necrosis, as the normal epithelium and submucosal lining are already disrupted by tumor tissue(1). Whether this is true in clinical practice has yet to be tested.

6.3. SAFETY

The results from Study 1 show that IRE in LAPC is associated with a risk of iatrogenic mortality and with several major and minor complications(1). The complication rates found in Study 1 were on par with the published studies at the time(124,136). The complications observed after IRE in LAPC can roughly be divided into infectious, bleeding, ischemic/thrombotic, pancreas-related and pain/general complications.

Infectious complications can be caused by micro- or fulminant perforation of the bowel or other viscera or via the introduction of bacteria to the peritoneum through the skin during needle insertion. Other infectious events include pneumonia, urinary tract infection or other infections related to anesthesia, hospital admission or related procedures. In Study 1, three patients experienced abscess formation, which theoretically delayed chemotherapy continuation(1). While most infectious complications are manageable, either by antibiotics or percutaneous drainage, bowel perforation is especially problematic, as its management often requires major surgery, which significantly prolongs convalescence, delays chemotherapy continuation, comes with a high risk of additional complications (e.g., peritoneal abscess formation, thrombosis, and pneumonia), and can be fatal(1,61).

Bleeding complications include intraperitoneal(1,61,62,77,137) and GI bleeding(1,61,138,139) and hematoma formation(64). Intraperitoneal bleeding was observed in two patients in Study 1(1). One patient had a minor self-limiting bleeding in the ablation zone, and a small aneurism of the GDA was discovered on a subsequent CT scan. The other had major intraabdominal bleeding, which was managed successfully by laparotomy, but fatal cases have been reported(61,62,137). While only a few reports of aneurisms or other intraabdominal bleeding incidents have been reported, they can be very serious and should be suspected if patients present with low blood pressure, anemia and/or unexplained abdominal pain. The mechanism for the development of these complications is currently unknown, but the suspected etiology could be heat-related, mechanical (needles passing through the artery) or directly related to the electroporation effect. It seems unlikely that patients would have preexisting undiscovered aneurisms, as all included patients in Study 1 had triple-phase contrast-enhanced CT scans prior to IRE(1). A common complication seen in several studies is bleeding upper GI ulcers(1,61,138,139). In Study 1, three cases of GI bleeding were observed within 90 days after IRE(1). Two out of three resolved without intervention, while the last required endoscopic intervention. Based on these

observations, as well as the observed cases of duodenal perforations, it seems likely that IRE in LAPC can cause ulcers to form. Ulcers may be caused either directly through the impact of electroporation on tissues that are included in the ablation area or indirectly by means of surgical stress or postoperative ulcerogenic medications, e.g., nonsteroidal anti-inflammatory drugs (NSAIDs). For both intraperitoneal and GI ulcers, it is probable that careful treatment planning and positioning of needle electrodes with good intraoperative imaging would alleviate or minimize the risk. It can be argued that percutaneous treatment of LAPC should, at a minimum, be carried out under CT guidance, as this would allow better three-dimensional awareness of needle placement and the ability to accurately confirm and document the needle arrangement prior to pulse delivery.

Ischemic/thrombotic complications include a narrowing or occlusion of vessels in the ablation zone and thrombosis due to other causes, i.e., deep vein thrombosis after prolonged immobility. In Study 1, one case of PV thrombosis was observed after IRE(1). This complication was successfully treated by low-molecular-weight heparin. In a large study of IRE used in the treatment of different malignancies, vessel patency was found to be affected by IRE in 4.4% of the treated lesions (n = 7 of 158)(140). Several studies specifically on LAPC have reported a narrowing of vessels or PV thrombosis(1,62,64,102,138,141). Two cases of liver failure due to PV and/or CHA occlusion have been reported(62,139). In a recent matched retrospective cohort study, patients treated with IRE and chemotherapy were compared to patients treated with radiotherapy and chemotherapy(102). The study found that patients treated with IRE did not have a higher proportion of overall thrombotic events (4 vs. 5, p=.89). However, the study was likely not powered to perform a proper statistical comparison of complications between the groups.

Pancreas-related complications encompass acute pancreatitis, pancreatic pseudocysts, pancreatic fistulas, and a loss of pancreatic function, i.e., exocrine pancreas insufficiency or diabetes. Acute pancreatitis and complications related to acute pancreatitis have been reported in many trials of IRE in PC(1,62,64,66–68,76). The severity varies widely. In Study 1, three cases of acute pancreatitis were registered(1). One resolved without complications, one developed a pancreatic pseudocyst, which was treated unsuccessfully, and one was determined to be secondary to microscopic perforation of the duodenum, which eventually led to abscess formation. Another common complication seen in Study 1 (n = 8 of 33) was exocrine pancreatic insufficiency(1). Although it was unclear whether a direct causality existed, it seems likely that the destruction of pancreatic tissue through IRE may cause (or exacerbate) insufficiency in the production and/or secretion of digestive enzymes. In either case, this complication is easily manageable in most cases.

Abdominal pain, nausea, muscle weakness, fatigue and other general adverse events were observed in several patients in Study 1(1). Perioperative pain was shown to be the worst immediately after the procedure but improved within hours in most patients.

A few patients experienced prolonged abdominal pain and other general symptoms. However, in most patients, these symptoms dissipated within the first month(142,143).

Overall, several important and potentially devastating complications have been seen in relation to IRE treatment of LAPC. The rate of major adverse events (CD grade ≥ 3) can be hard to definitively estimate because of differences in reporting and classifications among the published studies but was estimated to be approximately 21% in a recent systematic review(136). As mentioned in the preface to this section (Chapter 6), the rate and severity of the intervention must be seen in the context of efficacy and effect size. Unfortunately, the efficacy of IRE has not been definitively proven to date; thus, it is not possible to assess whether the potential benefit is worth the risk of complications. The efficacy of IRE in LAPC will be further discussed in section 6.4. An important point when assessing the complications observed after IRE is to compare the frequency of complications after IRE to the frequency of these complications in patients with PC in general, as well as in patients undergoing surgery, radiotherapy and/or chemotherapy. In any case, it is crucial to provide any potential candidate for IRE (whether in a clinical trial or for possible future clinical implementation) with the necessary information before joint decision-making. More information about risk factors for the development of complications would be highly useful when choosing the right treatment approach for a given patient. This is important for two reasons. First, IRE could do more harm than good in patients with high risk, and second, because patients with a low risk of complications should be able to pursue aggressive treatment, which has been demonstrated to bridge to surgical resection(1).

6.4. EFFICACY

In cancer research, there are different ways of determining the efficacy of an intervention. The gold standard is a comparison of OS in a well-designed RCT. In the absence of RCTs, preliminary indications of efficacy can be provided either by comparing the results to those of a control group in an unrandomized fashion or by a longitudinal comparison of established markers of response in a treated patient group (typically used in phase 1 and 2 trials). The latter can be especially relevant in cancers with long OS, where the follow-up time would be prohibitory. For any marker of response to be useful, it must be objective (to reduce the risk of bias), and it must be equally valid for both the intervention and control groups. In regard to PC, both RECIST(144) and CA-19-9(145) are valid markers of response during chemotherapy, but most studies still compare survival time, even in early-phase clinical trials (phases 1 and 2), as this is reachable without an unreasonably long follow-up time and because objective response (OR) as determined by both RECIST and CA-19-9 comes with important and clinically relevant caveats(144,146). Furthermore, it is much less clear

whether CA-19-9 response and especially imaging-based response evaluation strategies are valid markers of response after local ablative therapies in PC(2).

Study 2 showed that there is a marked diversity in the use and reporting of imaging methods across the field of pancreatic ablations(2). No studies included in the systematic review tested the correlation between survival and established standardized imaging response criteria. Thus, it is unknown whether these criteria are valid markers of response after IRE or other ablative therapies (further discussed in section 6.5).

This is problematic, as nearly all published studies were either uncontrolled or compared results to a matched control group of patients receiving chemotherapy. In addition to this problem, many of the studies published to date, including Study 1, have included patients after they had completed downsizing chemotherapy if R0 resection was still implausible based on imaging(1). This selection is important from an ethical standpoint because of the well-established efficacy of surgical resection, but it introduces a risk of bias in the selection process, as resectability can be very hard to determine from imaging after combination chemotherapy(147).

OS from different uncontrolled cohorts of IRE-treated patients cannot be objectively compared because of vast differences in selection criteria, the availability of combination chemotherapy, differing resectability criteria, standardized adjuvant therapies and more. Simply said, the stricter the selection criteria, the better the OS. Examples are the exclusion of patients with metastatic disease observed during laparotomy in open IRE or the requirement of stable disease after 3-4 months of chemotherapy prior to IRE(62,67). This selection problem is evident from studies that included CONSORT diagrams starting from the diagnosis of LAPC. One study found that from an initial cohort of 132 LAPC patients, 44 were eligible for IRE, 36 underwent exploratory laparotomy, and only 15 patients underwent the IRE procedure(139). In this small study, the efficacy of IRE could not be established, as patients who underwent laparotomy without resection or IRE (n = 7) displayed a longer median OS than patients in the IRE group (22 vs. 16 months). In comparison, all patients who had stable disease or better after restaging and went on to receive chemotherapy had a median OS of only 15 months. It is, however, not entirely clear from the article why IRE was not performed in some of the patients undergoing laparotomy, and thus selection bias cannot be ruled out.

Several matched studies have been published regarding the efficacy of IRE. In the first study, IRE- and chemotherapy-treated LAPC patients (n = 36) were matched to LAPC patients receiving chemotherapy only (n = 96) during the same time period(148). After propensity score matching (1:1), based on several parameters, IRE-treated patients had significantly higher 1- and 2-year survival rates than the chemotherapy-only group (p=.001). The median OS for the IRE group was not reached during the planned 24-month follow-up period per patient, whereas the median OS was only 7.1 months after 4 months of induction therapy in the

chemotherapy-only group. However, when examining the Kaplan–Meier curves of survival in the study, it is obvious that many more patients were censored in the IRE group than in the radiotherapy group, which could be due to insufficient follow-up time or attrition bias. In a second study by the same authors, the same cohort of 36 IRE-treated patients was matched (1:1) to LAPC patients receiving chemotherapy and conventional radiotherapy (67 Gy over 30 sessions) (n = 40) from the same hospital and time period(102). The study found a statistically significant survival advantage in the group of patients treated with IRE (median OS 21.6 vs. 10.6 months, p=.01). However, due to the retrospective nature of both studies(102,148), attribution bias cannot be ruled out. A similar post hoc analysis was published by other authors(149). In this study, two separate cohorts of FOLFIRINOX-treated LAPC patients, with or without concurrent IRE ablation, were matched based on the length of FOLFIRINOX therapy and tumor size. In this study, patients treated with IRE and FOLFIRINOX had a significant survival advantage (median OS 17.2 vs. 12.4 months, p=.05) and had a longer progression-free survival (PFS) (13.1 vs. 4.9 months, p<.001). However, as was seen in a previous study(148), more patients were censored in the early follow-up period in the IRE group, which may have biased the results.

Only one RCT of IRE in LAPC patients has been published to date(150). In this trial, 68 treatment-naïve patients were randomized to either gemcitabine alone or IRE with simultaneous gemcitabine followed by gemcitabine. In the study, it was found that patients in the IRE + gemcitabine group had a significantly longer median OS (19.8 vs. 9.3 months, p=.0001). Likewise, patients treated with IRE + gemcitabine had a significantly longer median PFS (8.3 vs. 4.7 months, p=.0001). A $\leq$ 50% decrease in CA-19-9 three months after IRE and tumor volume at baseline > 37 cm^3 was associated with a worse OS (hazard ratio (HR) 2.7, p=.032 and HR 2.4, p=.023, respectively). The researchers were not able to find any significant difference in the rate of any of the observed adverse events between the two groups. However, the study was likely not powered to do so for rare events. This study, while being the highest evidence-level study published to date, comes with a few caveats regarding the efficacy of IRE. First, because IRE was combined with gemcitabine (injected intravenously 30 mins prior to treatment), a synergetic ECT effect may have occurred (more on this can be found in section 6.6), thus making the results inappropriate to evaluate whether IRE is efficacious without simultaneous chemotherapy. Second, because gemcitabine is no longer considered first-line chemotherapy for patients with PS 0 and 1, it is not certain whether the results are valid in the context of modern multimodal treatment. Nevertheless, the results clearly demonstrate the therapeutic efficacy of combined IRE + gemcitabine, which is encouraging and should lead to further investigations.

In conclusion, several studies have tried to answer whether IRE is efficacious in LAPC management. One RCT has been published to date and shows that IRE is efficacious, but the results may not be representative of how many of the prior and ongoing trials are conducted because the procedure was combined with gemcitabine and because the

chemotherapeutic regimens in both groups are no longer first-line treatment in patients with good PS. When assessing the studies of unenhanced IRE, the comparative studies point to the therapy being efficacious but come with a risk of bias in selection and follow-up. The study with the least risk of selection bias showed negative findings but was underpowered(139). Larger and properly conducted RCTs with relevant control groups are needed to definitively evaluate the efficacy of IRE in LAPC. However, because IRE has been shown to downstage/downsize the tumors of some patients to a resectable disease stage, it is clear that the treatment may be beneficial in some patients(1). However, whether SBRT or other novel radiotherapy treatments may provide better bridging to surgery than IRE is not clear.

6.5. OBJECTIVE RESPONSE EVALUATION IN ABLATION-TREATED PANCREATIC CANCER

Imaging response evaluation in cancer treatment is important for two reasons. First, imaging response evaluation can be used in clinical trials to estimate efficacy in the absence of hard outcomes, i.e., OS. Second, imaging response evaluation is an important tool in clinical patient care to prognosticate whether a given therapy will have the desired effect; thus, it can guide therapy.

The OR based on postablative imaging can be very difficult to assess in LAPC(151), which is because morphological imaging, i.e., CT, US and nonfunctional MRI, cannot distinguish between living cancer cells and the dense desmoplastic stroma associated with PC. Thus, when scar tissue is formed after ablative intervention, the effects of the treatment on cancer cells is not clear. This difficulty is further accentuated because most ablative interventions aim to include a margin of healthy tissue in the ablated area, which may produce a larger appearance on subsequent images as new scar tissue is formed in previously healthy tissue.

Study 2 was conducted to examine the methodology of imaging assessment after local ablative therapies in LAPC treatment(2). The study found vast differences in the methods used across the included trials and in the reporting of key imaging parameters, e.g., the timing of scans, response criteria used and evaluation target (patient-level or lesion-level). Additionally, very few (n = 2 of 34) trials performed comparisons with the first postablative scan as recommended by the SIR(2). Some of the apparent heterogeneity observed in Study 2 may be explained by differences in the aim of the ablations. For instance, several of the trials of RFA aimed to partially ablate tumors only and therefore included short-term measures of ablative coverage with comparison to preablative images as an outcome. Other differences in the outcomes chosen by different research groups may be explained by the difference between thermal and nonthermal interventions(2). In early postablative images,

thermal ablation may cause the treated tissues to shrink, whereas IRE and ECT induce intracellular edema, which causes the ablated tissues to swell.

Another important observation made in Study 2 was that only lesion-level outcomes were reported in many trials, even in long-term follow-up(2). This method can be useful in assessing the innate efficacy of IRE or other ablative treatments but fails to consider that ablations in LAPC may inadvertently cause the dissemination of disease. Thus, it is crucial that such considerations be clearly stated if evaluations of lesion-level outcomes only are reported.

It is important to differentiate between two scenarios when assessing postablative images in general. The first scenario is to assess whether the tumor tissue is completely covered by the ablation and whether a complete eradication of cancer cells can be expected, i.e., technical efficacy. The second scenario is to assess the long-term effects of the treatment. The terminology of the latter is dependent on the outcomes of the ablative efficacy assessment. For apparent complete ablations (i.e., without residual untreated tumor tissue), later postablative lesion-level imaging assessments will be able to observe recurrence or no recurrence. For incomplete ablations (whether deliberate or not), tumors may theoretically be categorized as showing response (partial or complete), SD or PD. However, to determine this, the comparison scan should ideally be the first postablative scan to account for issues regarding the immediate effects of treatment, as previously described(85). To complicate things further, some studies of IRE have shown that the treatment may take effect over longer periods of time, up to two months(152,153), which significantly prolongs the interval during which the first postablative scan should be performed to assess technical efficacy.

Because RECIST was developed to assess the response to chemotherapy, it provides only minor guidance in the assessment of lesions with previous local treatment, i.e., that these should be categorized as nontarget lesions. This guidance is very useful in the context of metastatic disease, as other target lesions will be used to define response. However, in the context of LAPC, this becomes problematic, as nontarget lesions can be categorized only as showing complete response (CR) (i.e., the disappearance of lesions and normalization of tumor markers), non-CR/non-PD (i.e., persistence of nontarget lesion(s) and/or maintenance of tumor marker levels) or PD (i.e., unequivocal progression of existing nontarget lesion(s) or the appearance of new lesions)(86). Because of the problems regarding postablative fibrosis and assessment of efficacy, as mentioned above, the CR category is obsolete after IRE in LAPC; thus, lesions can be categorized only as PD or non-PD. Likewise, as was shown in Study 3, nearly all patients experienced local progression at some point after IRE or had residual disease in resected tumors(3). Therefore, efforts to distinguish between incomplete ablation and complete ablation in LAPC are not relevant, as it is clear that unenhanced IRE without subsequent surgery is not curative. Nevertheless, when evaluating a positive change after IRE, it is critical to examine whether radical

resection is likely. This evaluation is not easy for the same reasons observed in the context of combination chemotherapy(151). Based on this, it can be argued that patients without unequivocal local progression or metastatic progression should be considered for exploratory laparotomy after IRE.

In Study 3, the correlation between imaging response categories (PD or non-PD) and survival was examined, and it was discussed whether functional imaging using ^{18}FDG PET offered any advantages in evaluating response after IRE in PC(3). The main results were that patient-level PD based on RECIST and EORTC PET response criteria was correlated with a worse OS. The study was not able to find any objective advantages using PET scans, which may be due to the timing of PET/CT scans in the study because the first postablative scan was conducted after three months. However, it is also likely that the PET-based outcomes were influenced by inflammation in the ablation zone. The study was not able to find any significant correlation between lesion-level PD using different morphological and functional parameters. However, several of the examined lesion-level outcomes trended toward significance, meaning that these outcomes would likely become statistically significant in a larger trial(3). These outcomes were significantly correlated in subsequent statistical analysis without dichotomization. An overall trend of better distinction between PD and non-PD was noted when the first postablative scan was used as the baseline for comparison, which is in agreement with the recommendation made by the SIR(85).

6.6. IRREVERSIBLE ELECTROPORATION AND CHEMOTHERAPY

Combination treatment using IRE with simultaneous chemotherapy or calcium injections is highly feasible from a theoretical perspective, as these treatment principles have already been proven feasible in deep-seated cancers using conventional ECT(154–156). However, whether there is any advantage of using high PEF strengths, as is typical of IRE, over the lower PEF strengths used with RE is unknown. In addition, how a chemotherapeutic or other enhancing agent is distributed in the tissue during combined therapy with IRE is unknown. Computer simulations and in vitro models can provide some idea of the distribution based on the electrical field strength threshold of IRE and RE(57) but fail to consider the complex environment of real-world ablations, e.g., vessels running through the tumor and the heterogeneity of the stroma.

Study 4 was performed to assess and characterize the distribution of i.v. chemotherapy during IRE treatment in living animals (App. 4). Using the distribution of gadolinium contrast as a model for chemotherapy distribution, it was found that most of the contrast was distributed in a penumbra around the ablation zone, which effectively doubled the ablated volume (App. 4). This finding is consistent with those of other

studies regarding the RE zone observed after IRE and supports the hypothesis that the chemotherapeutic agent will be captured in cells that have reached the RE threshold but not in cells that have reached the IRE threshold. The reason for this is that IRE causes permanent permeabilization of the plasma membranes and thus allows for unrestricted diffusion of chemicals in both directions. In contrast, when pore formation is only transient, i.e., during RE, chemicals can be captured within the cells; thus, normal washout and metabolism of the chemical will not occur.

A clinically important observation made in Study 4 is that contrast enhancement was observed in the vicinity of vessels in the ablation zone, suggesting that these areas may be insufficiently electroporated to induce cell death during normal unenhanced IRE if the abovementioned hypothesis is true (App. 4). Based on this, it seems likely that combination treatment would be able to enhance the technical efficacy of IRE.

The choice of enhancing agent to combine with IRE is not clear cut. On the one hand, bleomycin and cisplatin are the preferred agents for ECT treatments because they have shown the largest increase in cytotoxicity when administered in combination with electroporation across different cancer histologies. On the other hand, these agents are not part of the standard treatment regimens in PC; therefore, it is moderately uncertain if the desired effect would emerge. CaEP is another option, as this is likely agnostic toward the histology of the cancer(55). However, as calcium injection cannot be performed intravenously, CaEP in PC would require either open surgery or alternatively EUS-guided i.t. injection to ensure proper injection in the lesion. Additionally, because acute pancreatitis can be caused by hypercalcemia, CaEP could, in theory, be associated with a high risk of acute pancreatitis.

In Study 4, it was not possible to detect bleomycin in the ablated area four hours after it was given as an i.v. injection in doses typically used for ECT (15,000 IU/m^2 BSA) (App. 4). The results from the subsequent pharmacokinetic study revealed that bleomycin was present in the blood at the timepoint when the ablations were performed. Therefore, the negative findings could be due to several issues. First, as bleomycin chelates to iron, which is abundant in the liver, it is possible that the analyses were not able to detect bleomycin in this tissue. Second, the storage and freezing protocol of the study could have affected the preservation or detectability of bleomycin. This possibility is, however, unlikely, as this was not different in the pharmacokinetic study. Third, the therapeutic index of bleomycin in the context of ECT may be so low that it is beyond the ability to detect using the available methods. Fourth, the hypothesis that a zone of RE is induced during IRE could be wrong. However, this possibility is contradicted by the contrast enhancement patterns observed in the study and by the findings of other studies, which have shown that the concentration of gemcitabine is increased after IRE(157) and that the effect of chemotherapy is increased when combined with IRE(158)

A few clinical trials on combining EP and chemotherapy in LAPC have been published to date, using either the conventional ECT approach(100,159) or IRE with chemotherapy enhancement(150,158). ECT with bleomycin has been evaluated in two smaller trials of LAPC (n = 13 and 5, respectively)(100,159). In both studies, the treatment was found to be feasible and without any major complications. In one study, the OS was not reached after a median follow-up time of 9.4 months from the intervention(159). In the other study, the survival time and follow-up time were not reported (100). Chemotherapy-enhanced IRE using i.v. FOLFIRINOX (n = 2) or gemcitabine (n = 3) was examined in a case series of five previously treated patients(158). Four patients were alive after a median follow-up of 9.4 months from the intervention. No major complications occurred after the intervention. The second trial was the RCT mentioned in the efficacy section (section 6.4)(150). In this trial, previously untreated patients were randomly assigned to receive gemcitabine-enhanced IRE followed by gemcitabine or gemcitabine only. The study displayed a clear benefit in survival by gemcitabine-enhanced IRE treatment (p=.0001). Regarding safety, the study showed no significant difference in the rate of overall adverse events between the groups. Two cases of acute pancreatitis and one case of a bleeding duodenal ulcer were observed in the IRE group and managed successfully. The reported results showed that gemcitabine-enhanced IRE was efficacious and had a clinically relevant effect size compared to gemcitabine alone. However, because no group of patients received unenhanced IRE, it cannot be definitively concluded whether this was the result of a synergistic effect or IRE ablation alone.

6.7. STRENGTHS AND LIMITATIONS

The presented studies have several strengths and limitations that should be highlighted.

6.7.1. STUDY 1

The small sample size and lack of a control group were major limiting factors in the determination of a causal relationship between a given adverse event and the intervention. The causal relationship with the procedure was especially hard to establish, as both the underlying disease and chemotherapy treatment are associated with a major risk of adverse events, which can be hard to distinguish. Therefore, all events in the first 90 days after the procedure were reported regardless of whether a causal relationship with the procedure was plausible. However, as the data were gathered prospectively and because of the ubiquitous access to patient charts across institutions in Denmark, the follow-up was excellent, with a very low chance of missing potential postoperative complications. The sample size was prohibitory in evaluating indicators for adverse events(1).

6.7.2. STUDY 2

The literature search was conducted in only one database, PubMed. Hence, there might be studies with valuable information that were missed by the search strategy. Nevertheless, the literature search was conducted with the aid of a research librarian, ensuring that the search terms were not overly narrow; additionally, previous publications have shown that a good search strategy in a single database can be superior to poor strategies conducted in multiple databases(160). Another limiting factor is that the selection criteria excluded studies with mixed patient cohorts, e.g., patients with metastatic PC or non-LAPC intermixed with LAPC. This selection method was chosen to ensure external validity in the context of LAPC; however, some of the excluded studies may have contained valuable information that could have contributed to the understanding of the field.

6.7.3. STUDY 3

As mentioned in the discussion, the sample size was prohibitory in establishing a statistical correlation between local imaging outcomes and survival. However, several trends were noticed that were statistically significant without dichotomization. Validation of the results in a larger population and ideally a randomized controlled population would be beneficial to ensure that imaging outcomes are valid in this context. In addition, because the first postablative images were obtained three months after the intervention, valuable information about the early postablative period may have been missed. This possibility is especially relevant, as a general trend of better differentiation between PD and non-PD was noticed when the first postablative images were used for comparison. Tumor attenuation outcomes, i.e., Choi response criteria, were not included in the analysis but may have been valuable in evaluating the response of IRE, as these would possibly be less affected by the immediate effects of the treatment.

6.7.4. STUDY 4

The major limitation in Study 4 was the inability to determine the exact reason why bleomycin was undetectable in the liver and pancreatic tissue. The negative finding of this study raises more questions than answers because clinical trials have found that bleomycin ECT is efficacious in liver and pancreatic tumors. Several possible reasons for this are discussed in App. 4. Moreover, because the experiment was performed in healthy, nontumor-bearing animals, it is not certain whether the observed inconsistencies in contrast enhancement are relevant in a cancer-treatment context. However, the study identifies a simple method for evaluating the RE zone, which is easily translatable into the clinic and could potentially be used for quality control of IRE ablations in LAPC or other unresectable tumors.

CHAPTER 7. CONCLUSIONS

In summary, IRE in LAPC carries a considerable risk of major and minor complications, which should be interpreted in the context of the risk profile of the candidates for treatment and the efficacy of the treatment. Several studies indicate that IRE is efficacious, but no definitive evidence is available to date(1).

Many studies have used OR based on imaging as an efficacy endpoint with little to no consensus between studies. Evidence for some practices in published studies is lacking, especially the use of lesion-level outcomes(2).

Using data derived from a cohort of IRE-treated patients with nonmetastatic PC, patient-level imaging response was correlated with survival, whereas lesion-level response evaluation was not significantly correlated. Several promising outcomes were identified. Functional imaging did not offer any advantages for differentiating progressors from nonprogressors(3).

The RE zone after IRE was easily visualizable using standard methods. These methods may be useful for quality control in clinical trials. Chemotherapeutic enhancement of IRE is likely to have a synergistic effect, but the choice of enhancing agent and timing should be carefully considered (App. 4).

CHAPTER 8. PERSPECTIVES

While the efficacy of unenhanced IRE is still disputed, many different centers have acquired the equipment to perform IRE, and many have published papers describing smaller uncontrolled cohorts of IRE-treated patients, such as Study 1(1). The complexity of multimodal treatment and management of LAPC is prohibitory in conducting single-center RCTs in all but the largest centers. However, multicenter RCTs are currently being conducted (https://www.clinicaltrials.gov/, NCT02791503 & NCT03899636).

Chemical enhancement represents an obvious evolution of the currently unenhanced IRE treatments. The findings from few studies that have been conducted to date are encouraging and show that combinations with proven chemotherapies, conventionally used in PC treatment, are feasible and safe(150,158). The choice of enhancing agent can be based on the following: 1) the use of chemotherapy agents with proven efficacy in PC; 2) the use of chemotherapy agents that have been proven to increase efficacy with EP during ECT, e.g., bleomycin or cisplatin; and 3) the combination of IRE with nonchemotherapeutic enhancing agents, i.e., CaEP.

To date, the only trial investigating the efficacy after enhanced IRE has been conducted using the first approach(150). However, as mentioned in the discussion (section 6.6), it is not possible to clearly conclude whether the results of this study were due to a synergistic effect, as no group received unenhanced IRE. However, it seems highly likely that this approach is valid, as other researchers have found gemcitabine to be captured in IRE-ablated areas(157). Gemcitabine-enhanced IRE is currently being compared with unenhanced IRE in an RCT (https://www.clinicaltrials.gov/, NCT03673137).

Whether the other enhancement approaches are valid is a subject for future trials. However, as shown in Study 4, i.v. bleomycin may not be the enhancement method of choice based on the inability to detect it in pancreatic or liver tissue after bleomycin-enhanced IRE (App. 4).

A recent discovery was that IRE has immune-response-promoting effects(161), which in turn may relieve resistance to checkpoint inhibition (CPI)(162). This possibility is especially enticing in the context of PC, as all the published clinical trials of CPIs in PC treatment have been negative(163–166). Several potential mechanisms contribute to the lack of an effect of immunotherapy in PC(167). However, in a recent preclinical study, the combination of nivolumab (a PD1 antibody) and IRE was tested in a murine PC model(162). In the study, the combination of nivolumab and IRE resulted in a significantly prolonged median OS (31.5 days, $p<.0001$) compared to IRE alone (11.5 days), nivolumab alone (8 days) and no treatment (6 days). Approximately 40% of the mice treated with the combination were found to be tumor free at the end of the trial,

and all rejected rechallenging inoculation with new tumor cells, which indicates that long-term T cell memory was established. In a subsequent substudy, the researchers compared IRE + nivolumab with radiation therapy (10 Gy) + nivolumab. In this experiment, the mOS was similar, but all the radiation-treated mice had progression and died by Day 55. In contrast, 4 of 11 mice in the IRE group survived for 120 days. Other researchers have found a similar positive effect when combined with other and less clinically adopted immunotherapeutic agents(168–170). The mechanisms for the immune-stimulating properties of IRE are not definitively known. The immune-stimulating properties of IRE may be a result of the type of cellular death induced by IRE(171,172), the increased release of cancer-specific proteins(173), danger-associated molecular patterns (DAMPs) after IRE(162,172), other undiscovered mechanisms or a combination of several factors.

To the authors' knowledge, only three clinical trials examining the combination of IRE and immunotherapy in PC have been published to date (74,174,175). The first is a phase 1b uncontrolled trial in ten LAPC patients receiving IRE + nivolumab(174). In this trial, a median OS of 18 months was observed, and no dose-limiting toxicities were observed. The second study was a retrospective comparison of 70 LAPC patients receiving IRE only and 15 LAPC patients receiving IRE + toripalimab(175). The study arguably did not have enough follow-up time in the IRE + toripalimab group to estimate efficacy. However, the study results showed that the combination was feasible and that no treatment-related death occurred in the follow-up period. The study additionally compared immune cells isolated from peripheral blood before and after treatment in both groups. The concentrations of CD4+ T cells and CD8+ T cells and the CD8+/CD4+ ratio increased during treatment with IRE (before vs. after). However, these observations were significantly more pronounced in the IRE + toripalimab group. In addition, a decrease in regulatory T cells and increased levels of interferon-gamma and tumor necrosis factor-beta were observed. The third study evaluated an unrandomized prospective cohort of LAPC and oligometastatic PC patients treated with IRE only or in combination with allogenic natural killer cell transplantation(74). A survival advantage was found in the combination arm for both LAPC and oligometastatic patients (p=.0411 and p=.0397, respectively). Similar to a previous study(175), this study found periprocedural increases in the peripheral blood concentrations of adaptive immune cells (CD4+ and CD8+) as well as the levels of interferon-gamma and tumor necrosis factor-beta(74).

These findings are encouraging and suggest that IRE may be able to potentiate CPIs and other immunotherapies in PC and other cancers that have not responded to CPIs thus far. The importance of finding a way to elicit an anticancer immune response cannot be overstated, as this would open the door for treatments for both patients with LAPC and the many patients with metastatic PC by a mechanism of action that is completely different from that of conventional chemotherapy. This would allow an additional line of treatment and a different toxicity profile from that of chemotherapy. Based on the findings above, our group has designed and is currently conducting a

phase 2 trial of combined pembrolizumab and IRE in liver metastatic PC (https://www.clinicaltrials.gov/, NCT04835402). Other research groups are currently conducting similar clinical trials of combined immunotherapy and IRE in PC (https://www.clinicaltrials.gov/, NCT03080974, NCT04612530 & NCT04212026).

REFERENCES

1. Flak RV, Stender MT, Jensen TM, Andersen KL, Henriksen SD, Mortensen PB, et al. Treatment of locally advanced pancreatic cancer with irreversible electroporation – a Danish single center study of safety and feasibility. Scand J Gastroenterol. 2019 Feb 1;54(2):252–8.

2. Flak RV, Stender MT, Stenholt L, Thorlacius-Ussing O, Petersen LJ. Imaging response evaluation after local ablative treatments in locally advanced pancreatic cancer: an expedited systematic review. HPB. 2020 Aug;22(8):1083–91.

3. Flak R V., Fisker R V., Bruun NH, Stender MT, Thorlacius-Ussing O, Petersen LJ. Usefulness of Imaging Response Assessment after Irreversible Electroporation of Localized Pancreatic Cancer—Results from a Prospective Cohort. Cancers (Basel). 2021 Jun 8;13(12):2862.

4. Yu J, Yang X, He W, Ye W. Burden of pancreatic cancer along with attributable risk factors in Europe between 1990 and 2019, and projections until 2039. Int J Cancer. 2021 Sep 1;149(5):993–1001.

5. Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. Lancet. 2020 Jun;395(10242):2008–20.

6. Tanaka M, Mihaljevic AL, Probst P, Heckler M, Klaiber U, Heger U, et al. Meta-analysis of recurrence pattern after resection for pancreatic cancer. Br J Surg. 2019 Oct 22;106(12):1590–601.

7. World Health Organization. Global Cancer Observatory [Internet]. 2020 [cited 2021 Sep 21]. Available from: https://gco.iarc.fr/

8. Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Nikšić M, et al. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet. 2018 Mar 17;391(10125):1023–75.

9. Rasmussen LS, Fristrup CW, Jensen B V., Pfeiffer P, Weber B, Yilmaz MK, et al. Initial treatment and survival in 4163 Danish patients with pancreatic cancer: A nationwide unselected real-world register study. Eur J Cancer. 2020 Apr 1;129:50–9.

10. Schmidt-Hansen M, Berendse S, Hamilton W. Symptoms of Pancreatic Cancer in Primary Care. Pancreas. 2016 Jul;45(6):814–8.

11. The Danish Health Authority. Pakkeforløb for kræft i bugspytkirtel, galdegange og lever [Internet]. [cited 2021 Oct 20]. Available from: https://www.sst.dk/da/Udgivelser/2021/Pakkeforloeb-for-kraeft-i-bugspytkirtel_-galdegange-og-lever

12. Dansk Pancreas Cancer Gruppe (DPCG). Nationale Kliniske Retningslinier - Pancreas Cancer [Internet]. [cited 2018 Oct 10]. Available from: http://dpcg.gicancer.dk/Default.aspx?pID=22

13. Pereira SP, Oldfield L, Ney A, Hart PA, Keane MG, Pandol SJ, et al. Early detection of pancreatic cancer. Lancet Gastroenterol Hepatol. 2020 Jul 1;5(7):698–710.

14. Bosetti C, Lucenteforte E, Silverman DT, Petersen G, Bracci PM, Ji BT, et al. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). Ann Oncol. 2012 Jul;23(7):1880–8.

15. Duell EJ, Lucenteforte E, Olson SH, Bracci PM, Li D, Risch HA, et al. Pancreatitis and pancreatic cancer risk: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Ann Oncol. 2012 Nov;23(11):2964–70.

16. Ryan DP, Hong TS, Bardeesy N. Pancreatic Adenocarcinoma. N Engl J Med. 2014 Sep 11;371(11):1039–49.

17. Yeo TP. Demographics, Epidemiology, and Inheritance of Pancreatic Ductal Adenocarcinoma. Semin Oncol. 2015 Feb;42(1):8–18.

18. Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. Clin Cancer Res. 2000 Aug;6(8):2969–72.

19. Notta F, Chan-Seng-Yue M, Lemire M, Li Y, Wilson GW, Connor AA, et al. A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns. Nature. 2016 Oct 20;538(7625):378–82.

20. Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al., editors. AJCC Cancer Staging Manual. AJCC Cancer Staging Manual. Cham: Springer International Publishing; 2017.

21. Tempero MA. NCCN Guidelines Updates: Pancreatic Cancer. Vol. 17, Journal of the National Comprehensive Cancer Network : JNCCN. 2019. p. 603–5.

22. Loizou L, Duran CV, Axelsson E, Andersson M, Keussen I, Strinnholm J, et al. Radiological assessment of local resectability status in patients with pancreatic cancer: Interreader agreement and reader performance in two different classification systems. Eur J Radiol. 2018 Sep;106:69–76.

23. Katz MHG, Varadhachary GR, Fleming JB, Wolff RA, Lee JE, Pisters PWT, et al. Serum CA 19-9 as a Marker of Resectability and Survival in Patients with Potentially Resectable Pancreatic Cancer Treated with Neoadjuvant Chemoradiation. Ann Surg Oncol. 2010 Jul 17;17(7):1794–801.

24. Santucci N, Facy O, Ortega-Deballon P, Lequeu J-B, Rat P, Rat P. CA 19–9 predicts resectability of pancreatic cancer even in jaundiced patients. Pancreatology. 2018 Sep 1;18(6):666–70.

25. Versteijne E, Suker M, Groothuis K, Akkermans-Vogelaar JM, Besselink MG, Bonsing BA, et al. Preoperative Chemoradiotherapy Versus Immediate Surgery for Resectable and Borderline Resectable Pancreatic Cancer: Results of the Dutch Randomized Phase III PREOPANC Trial. J Clin Oncol. 2020 Jun 1;38(16):1763–73.

26. Hogendorf P, Skulimowski A, Durczyński A, Kumor A, Poznańska G, Poznańska A, et al. Elevated preoperative levels of CA 19-9 and CA 125 predicts overall survival time in the pancreatic adenocarcinoma. Single institution series. Polish J Surg. 2020 Apr 16;92(3):1–5.

27. Brown EG, Canter RJ, Bold RJ. Preoperative CA 19-9 kinetics as a prognostic variable in radiographically resectable pancreatic adenocarcinoma. J Surg Oncol. 2015 Mar 1;111(3):293–8.

28. Isaji S, Mizuno S, Windsor JA, Bassi C, Fernández-del Castillo C, Hackert T, et al. International consensus on definition and criteria of borderline resectable pancreatic ductal adenocarcinoma 2017. Pancreatology. 2018 Jan;18(1):2–11.

29. Ye M, Zhang Q, Chen Y, Fu Q, Li X, Bai X, et al. Neoadjuvant chemotherapy for primary resectable pancreatic cancer: a systematic review and meta-analysis. HPB. 2020 Jun;22(6):821–32.

30. Artinyan A, Soriano PA, Prendergast C, Low T, Ellenhorn JDI, Kim J. The anatomic location of pancreatic cancer is a prognostic factor for survival. HPB. 2008 Oct;10(5):371–6.

31. Delpero JR, Sauvanet A. Vascular Resection for Pancreatic Cancer: 2019 French Recommendations Based on a Literature Review From 2008 to 6-

2019. Front Oncol. 2020 Feb 4;10:40.

32. Jegatheeswaran S, Baltatzis M, Jamdar S, Siriwardena AK. Superior mesenteric artery (SMA) resection during pancreatectomy for malignant disease of the pancreas: a systematic review. HPB. 2017 Jun;19(6):483–90.

33. Schlitter AM, Esposito I. Definition of Microscopic Tumor Clearance (R0) in Pancreatic Cancer Resections. Cancers (Basel). 2010 Nov 25;2(4):2001–10.

34. Sun Y-M, Wang Y, Mao Y-X, Wang W. The Safety and Feasibility of Enhanced Recovery after Surgery in Patients Undergoing Pancreaticoduodenectomy: An Updated Meta-Analysis. Biomed Res Int. 2020 May 9;2020:1–15.

35. Dansk Pancreas Cancer Database (DPCD). Årsrapport 2017/2018. 2019.

36. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer. N Engl J Med. 2011 May 12;364(19):1817–25.

37. Pusceddu S, Ghidini M, Torchio M, Corti F, Tomasello G, Niger M, et al. Comparative Effectiveness of Gemcitabine plus Nab-Paclitaxel and FOLFIRINOX in the First-Line Setting of Metastatic Pancreatic Cancer: A Systematic Review and Meta-Analysis. Cancers (Basel). 2019 Apr 5;11(4):484.

38. Damm M, Efremov L, Birnbach B, Terrero G, Kleeff J, Mikolajczyk R, et al. Efficacy and Safety of Neoadjuvant Gemcitabine Plus Nab-Paclitaxel in Borderline Resectable and Locally Advanced Pancreatic Cancer—A Systematic Review and Meta-Analysis. Cancers (Basel). 2021 Aug 27;13(17):4326.

39. Khushman M, Dempsey N, Cudris Maldonado J, Loaiza-Bonilla A, Velez M, Carcas L, et al. Full dose neoadjuvant FOLFIRINOX is associated with prolonged survival in patients with locally advanced pancreatic adenocarcinoma. Pancreatology. 2015 Nov 1;15(6):667–73.

40. Versteijne E, Vogel JA, Besselink MG, Busch ORC, Wilmink JW, Daams JG, et al. Meta-analysis comparing upfront surgery with neoadjuvant treatment in patients with resectable or borderline resectable pancreatic cancer. Br J Surg. 2018 Jun 14;105(8):946–58.

41. Oba A, Ho F, Bao QR, Al-Musawi MH, Schulick RD, Del Chiaro M. Neoadjuvant Treatment in Pancreatic Cancer. Front Oncol. 2020 Feb

28;10:245.

42. Iyengar S, Nevala-Plagemann C, Garrido-Laguna I. Updates on adjuvant and neoadjuvant treatment strategies for surgically resectable and borderline resectable pancreatic ductal adenocarcinoma. Ther Adv Med Oncol. 2021 Jan 18;13:175883592110458.

43. Grossberg AJ, Chu LC, Deig CR, Fishman EK, Hwang WL, Maitra A, et al. Multidisciplinary standards of care and recent progress in pancreatic ductal adenocarcinoma. CA Cancer J Clin. 2020 Sep 19;70(5):375–403.

44. Neoptolemos JP, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: current and future perspectives. Nat Rev Gastroenterol Hepatol. 2018 Jun 1;15(6):333–48.

45. Huguet F, Dabout V, Rivin del Campo E, Gaujoux S, Bachet JB. The role of radiotherapy in locally advanced pancreatic cancer. Br J Radiol. 2021 Sep 1;94(1125):20210044.

46. Liu S, Liu Y, Yang J, Santos T Dos, Yang L, Li M, et al. Survival outcome after stereotactic body radiotherapy for locally advanced and borderline resectable pancreatic cancer: A systematic review and meta-analysis. Transl Oncol. 2021 Aug 1;14(8):101139.

47. Gehl J. Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. Acta Physiol Scand. 2003 Apr;177(4):437–47.

48. Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH. Gene transfer into mouse lyoma cells by electroporation in high electric fields. EMBO J. 1982 Jul;1(7):841–5.

49. Belehradek M, Domenge C, Luboinski B, Orlowski S, Belehradek J, Mir LM. Electrochemotherapy, a new antitumor treatment. First clinical phase I-II trial. Cancer. 1993 Dec 15;72(12):3694–700.

50. National Institute for Health and Clinical Excellence (NICE). Electrochemotherapy for metastases in the skin from tumours of non-skin origin and melanoma. 2013.

51. Jaroszeski MJ, Dang V, Pottinger C, Hickey J, Gilbert R, Heller R. Toxicity of anticancer agents mediated by electroporation in vitro. Anticancer Drugs. 2000 Mar;11(3):201–8.

52. Miklavčič D, Serša G, Brecelj E, Gehl J, Soden D, Bianchi G, et al.

Electrochemotherapy: technological advancements for efficient electroporation-based treatment of internal tumors. Med Biol Eng Comput. 2012 Dec 21;50(12):1213–25.

53. Gehl J, Sersa G, Matthiessen LW, Muir T, Soden D, Occhini A, et al. Updated standard operating procedures for electrochemotherapy of cutaneous tumours and skin metastases. Acta Oncol (Madr). 2018 Jul 3;57(7):874–82.

54. Rudno-Rudzińska J, Kielan W, Guziński M, Płochocki M, Antończyk A, Kulbacka J. New therapeutic strategy: Personalization of pancreatic cancer treatment-irreversible electroporation (IRE), electrochemotherapy (ECT) and calcium electroporation (CaEP) – A pilot preclinical study. Surg Oncol. 2021 Sep 1;38:101634.

55. Frandsen SK, Vissing M, Gehl J. A Comprehensive Review of Calcium Electroporation—A Novel Cancer Treatment Modality. Cancers (Basel). 2020 Jan 25;12(2):290.

56. Rubinsky B. Irreversible Electroporation in Medicine. Technol Cancer Res Treat. 2007 Aug 24;6(4):255–9.

57. Davalos R V., Mir LM, Rubinsky B. Tissue Ablation with Irreversible Electroporation. Ann Biomed Eng. 2005 Feb;33(2):223–31.

58. Zorbas G, Samaras T. A study of the sink effect by blood vessels in radiofrequency ablation. Comput Biol Med. 2015 Feb;57(2015):182–6.

59. Pillai K, Akhter J, Chua TC, Shehata M, Alzahrani N, Al-Alem I, et al. Heat Sink Effect on Tumor Ablation Characteristics as Observed in Monopolar Radiofrequency, Bipolar Radiofrequency, and Microwave, Using Ex Vivo Calf Liver Model. Medicine (Baltimore). 2015 Mar;94(9):e580.

60. Martin RCG. Multi-disciplinary management of locally advanced pancreatic cancer with irreversible electroporation. J Surg Oncol. 2017 Jul;116(1):35–45.

61. Kluger MD, Epelboym I, Schrope BA, Mahendraraj K, Hecht EM, Susman J, et al. Single-Institution Experience with Irreversible Electroporation for T4 Pancreatic Cancer: First 50 Patients. Ann Surg Oncol. 2016 May 29;23(5):1736–43.

62. Martin RCG, Kwon D, Chalikonda S, Sellers M, Kotz E, Scoggins C, et al. Treatment of 200 Locally Advanced (Stage III) Pancreatic Adenocarcinoma Patients With Irreversible Electroporation. Ann Surg. 2015 Sep;262(3):486–

94.

63. Lambert L, Horejs J, Krska Z, Hoskovec D, Petruzelka L, Krechler T, et al. Treatment of locally advanced pancreatic cancer by percutaneous and intraoperative irreversible electroporation: general hospital cancer center experience. Neoplasma. 2016 Jan 16;63(2):269–73.

64. Narayanan G, Hosein PJ, Beulaygue IC, Froud T, Scheffer HJ, Venkat SR, et al. Percutaneous Image-Guided Irreversible Electroporation for the Treatment of Unresectable, Locally Advanced Pancreatic Adenocarcinoma. J Vasc Interv Radiol. 2017 Mar;28(3):342–8.

65. Månsson C, Brahmstaedt R, Nilsson A, Nygren P, Karlson B-M. Percutaneous irreversible electroporation for treatment of locally advanced pancreatic cancer following chemotherapy or radiochemotherapy. Eur J Surg Oncol. 2016 Sep;42(9):1401–6.

66. Venkat S, Hosein PJ, Narayanan G. Percutaneous Approach to Irreversible Electroporation of the Pancreas: Miami Protocol. Tech Vasc Interv Radiol. 2015 Sep;18(3):153–8.

67. Leen E, Picard J, Stebbing J, Abel M, Dhillon T, Wasan H. Percutaneous irreversible electroporation with systemic treatment for locally advanced pancreatic adenocarcinoma. J Gastrointest Oncol. 2018 Apr;9(2):275–81.

68. Scheffer HJ, Vroomen LGPH, de Jong MC, Melenhorst MCAM, Zonderhuis BM, Daams F, et al. Ablation of Locally Advanced Pancreatic Cancer with Percutaneous Irreversible Electroporation: Results of the Phase I/II PANFIRE Study. Radiology. 2017 Feb;282(2):585–97.

69. Arena CB, Sano MB, Rossmeisl JH, Caldwell JL, Garcia PA, Rylander MN, et al. High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction. Biomed Eng Online. 2011 Dec 21;10(1):102.

70. Dong S, Wang H, Zhao Y, Sun Y, Yao C. First Human Trial of High-Frequency Irreversible Electroporation Therapy for Prostate Cancer. Technol Cancer Res Treat. 2018 Jan 25;17:153303381878969.

71. LORENZO MF, ARENA CB, DAVALOS R V. Maximizing Local Access to Therapeutic Deliveries in Glioblastoma. Part III: Irreversible Electroporation and High-Frequency Irreversible Electroporation for the Eradication of Glioblastoma. In: Glioblastoma. Codon Publications; 2017. p. 373–93.

72. National Institute for Health and Clinical Excellence (NICE). Irreversible electroporation for treating pancreatic cancer. 2017.

73. Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, et al. DPC4 Gene Status of the Primary Carcinoma Correlates With Patterns of Failure in Patients With Pancreatic Cancer. J Clin Oncol. 2009 Apr 10;27(11):1806–13.

74. Lin M, Alnaggar M, Liang S, Wang X, Liang Y, Zhang M, et al. An important discovery on combination of irreversible electroporation and allogeneic natural killer cell immunotherapy for unresectable pancreatic cancer. Oncotarget. 2017 Nov 24;8(60):101795–807.

75. Narayanan G, Hosein PJ, Arora G, Barbery KJ, Froud T, Livingstone AS, et al. Percutaneous Irreversible Electroporation for Downstaging and Control of Unresectable Pancreatic Adenocarcinoma. J Vasc Interv Radiol. 2012 Dec;23(12):1613–21.

76. Månsson C, Bergenfeldt M, Brahmstaedt R, Karlson B-M, Nygren P, Nilsson A. Safety and preliminary efficacy of ultrasound-guided percutaneous irreversible electroporation for treatment of localized pancreatic cancer. Anticancer Res. 2014 Jan;34(1):289–93.

77. Huang K-W, Yang P-C, Pua U, Kim M-D, Li S-P, Qiu Y-D, et al. The efficacy of combination of induction chemotherapy and irreversible electroporation ablation for patients with locally advanced pancreatic adenocarcinoma. J Surg Oncol. 2018 Jul 7;118(1):31–6.

78. Kwon D, McFarland K, Velanovich V, Martin RCG. Borderline and locally advanced pancreatic adenocarcinoma margin accentuation with intraoperative irreversible electroporation. Surgery. 2014 Oct;156(4):910–22.

79. Dindo D, Demartines N, Clavien P-A. Classification of Surgical Complications. Ann Surg. 2004 Aug;240(2):205–13.

80. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, et al. The Clavien-Dindo Classification of Surgical Complications. Ann Surg. 2009 Aug;250(2):187–96.

81. PRISMA [Internet]. [cited 2019 Dec 20]. Available from: http://www.prisma-statement.org/

82. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting

systematic reviews. BMJ. 2021 Mar 29;372:n71.

83. Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia [Internet]. Available from: www.covidence.org

84. Boellaard R, Delgado-Bolton R, Oyen WJG, Giammarile F, Tatsch K, Eschner W, et al. FDG PET/CT: EANM procedure guidelines for tumour imaging: version 2.0. Eur J Nucl Med Mol Imaging. 2015 Feb 2;42(2):328–54.

85. Ahmed M, Solbiati L, Brace CL, Breen DJ, Callstrom MR, Charboneau JW, et al. Image-Guided Tumor Ablation: Standardization of Terminology and Reporting Criteria—A 10-Year Update. J Vasc Interv Radiol. 2014 Nov;25(11):1691-1705.e4.

86. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228–47.

87. Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, Lammertsma AA, et al. Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. Eur J Cancer. 1999 Dec;35(13):1773–82.

88. Zou G. A Modified Poisson Regression Approach to Prospective Studies with Binary Data. Am J Epidemiol. 2004 Apr 1;159(7):702–6.

89. Martin RCG, Durham AN, Besselink MG, Iannitti D, Weiss MJ, Wolfgang CL, et al. Irreversible electroporation in locally advanced pancreatic cancer: A call for standardization of energy delivery. J Surg Oncol. 2016 Dec;114(7):865–71.

90. Kjaergaard B, Zepernick PR, Bergmann A, Jensen HK, Mladenovic M, Rasmussen BS. CT-guided needle lung biopsy is possible during apneic oxygenation: a case series. Multidiscip Respir Med. 2013 Dec 6;8(1):73.

91. Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin J-C, Pujol S, et al. 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magn Reson Imaging. 2012 Nov;30(9):1323–41.

92. 3D Slicer image computing platform [Internet]. [cited 2021 Jul 8]. Available from: https://www.slicer.org/

93. Vogl TJ, Panahi B, Albrecht MH, Naguib NNN, Nour-Eldin N-EA, Gruber-

Rouh T, et al. Microwave ablation of pancreatic tumors. Minim Invasive Ther Allied Technol. 2018 Jan 2;27(1):33–40.

94. Liu S, Qin Z, Xu J, Zeng J, Chen J, Niu L, et al. Irreversible electroporation combined with chemotherapy for unresectable pancreatic carcinoma: a prospective cohort study. Onco Targets Ther. 2019 Feb;Volume 12:1341–50.

95. Gao H-F, Wang K, Meng Z-Q, Chen Z, Lin J-H, Zhou Z-H, et al. High intensity focused ultrasound treatment for patients with local advanced pancreatic cancer. Hepatogastroenterology. 2013;60:26–9.

96. Vidal-Jove J, Perich E, del Castillo MA. Ultrasound Guided High Intensity Focused Ultrasound for malignant tumors: The Spanish experience of survival advantage in stage III and IV pancreatic cancer. Ultrason Sonochem. 2015 Nov;27(January 2010):703–6.

97. Yan L, Chen Y-L, Su M, Liu T, Xu K, Liang F, et al. A Single-institution Experience with Open Irreversible Electroporation for Locally Advanced Pancreatic Carcinoma. Chin Med J (Engl). 2016 Dec 20;129(24):2920–5.

98. Anzidei M, Marincola BC, Bezzi M, Brachetti G, Nudo F, Cortesi E, et al. Magnetic Resonance–Guided High-Intensity Focused Ultrasound Treatment of Locally Advanced Pancreatic Adenocarcinoma. Invest Radiol. 2014 Dec;49(12):759–65.

99. Granata V, Fusco R, Setola SV, Piccirillo M, Leongito M, Palaia R, et al. Early radiological assessment of locally advanced pancreatic cancer treated with electrochemotherapy. World J Gastroenterol. 2017 Jul 14;23(26):4767.

100. Granata V, Fusco R, Piccirillo M, Palaia R, Petrillo A, Lastoria S, et al. Electrochemotherapy in locally advanced pancreatic cancer: Preliminary results. Int J Surg. 2015 Jun 1;18:230–6.

101. Granata V, Fusco R, Setola SV, Palaia R, Albino V, Piccirillo M, et al. Diffusion kurtosis imaging and conventional diffusion weighted imaging to assess electrochemotherapy response in locally advanced pancreatic cancer. Radiol Oncol. 2019 Jan 19;53(1):15–24.

102. He C, Wang J, Sun S, Zhang Y, Lin X, Lao X, et al. Irreversible electroporation versus radiotherapy after induction chemotherapy on survival in patients with locally advanced pancreatic cancer: a propensity score analysis. BMC Cancer. 2019 Dec 27;19(1):394.

103. Li Y-J, Huang G-L, Sun X-L, Zhao X-C, Li Z-G. The combination therapy of

high-intensity focused ultrasound with radiotherapy in locally advanced pancreatic carcinoma. World J Surg Oncol. 2016 Dec 29;14(1):60.

104. Lin M, Xie X, Xu M, Feng S, Tian W, Zhuang B, et al. Non-enhanced Pattern on Contrast-Enhanced Ultrasound in the Local Efficacy Assessment of Irreversible Electroporation Ablation of Pancreatic Adenocarcinoma. Ultrasound Med Biol. 2018 Sep 1;44(9):1986–95.

105. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET Response Criteria in Solid Tumors. J Nucl Med. 2009 May;50(Suppl 1):122S-150S.

106. Choi H, Charnsangavej C, Faria SC, Macapinlac HA, Burgess MA, Patel SR, et al. Correlation of Computed Tomography and Positron Emission Tomography in Patients With Metastatic Gastrointestinal Stromal Tumor Treated at a Single Institution With Imatinib Mesylate: Proposal of New Computed Tomography Response Criteria. J Clin Oncol. 2007 May 1;25(13):1753–9.

107. Lencioni R, Llovet J. Modified RECIST (mRECIST) Assessment for Hepatocellular Carcinoma. Semin Liver Dis. 2010 Feb 19;30(01):052–60.

108. Belfiore G, Belfiore MP, Reginelli A, Capasso R, Romano F, Ianniello G Pietro, et al. Concurrent chemotherapy alone versus irreversible electroporation followed by chemotherapy on survival in patients with locally advanced pancreatic cancer. Med Oncol. 2017 Mar 4;34(3):38.

109. Carrafiello G, Ierardi AM, Fontana F, Petrillo M, Floridi C, Lucchina N, et al. Microwave Ablation of Pancreatic Head Cancer: Safety and Efficacy. J Vasc Interv Radiol. 2013 Oct;24(10):1513–20.

110. D'Onofrio M, Crosara S, De Robertis R, Butturini G, Salvia R, Paiella S, et al. Percutaneous Radiofrequency Ablation of Unresectable Locally Advanced Pancreatic Cancer: Preliminary Results. Technol Cancer Res Treat. 2017 Jun 18;16(3):285–94.

111. Ierardi AM, Biondetti P, Coppola A, Fumarola EM, Biasina AM, Alessio Angileri S, et al. Percutaneous microwave thermosphere ablation of pancreatic tumours. Gland Surg. 2018 Apr;7(2):59–66.

112. Zhao H, Yang G, Wang D, Yu X, Zhang Y, Zhu J, et al. Concurrent gemcitabine and high-intensity focused ultrasound therapy in patients with locally advanced pancreatic cancer. Anticancer Drugs. 2010 Apr;21(4):447–52.

113. D'Onofrio M, Barbi E, Girelli R, Martini PT, De Robertis R, Ciaravino V, et al. Variation of tumoral marker after radiofrequency ablation of pancreatic adenocarcinoma. J Gastrointest Oncol. 2015 Apr;7(2):213–20.

114. Rombouts SJE, Derksen TC, Nio CY, van Hillegersberg R, van Santvoort HC, Walma MS, et al. Computed tomography findings after radiofrequency ablation in locally advanced pancreatic cancer. Abdom Radiol. 2018 Oct 28;43(10):2702–11.

115. Scopelliti F, Pea A, Conigliaro R, Butturini G, Frigerio I, Regi P, et al. Technique, safety, and feasibility of EUS-guided radiofrequency ablation in unresectable pancreatic cancer. Surg Endosc. 2018 Sep 15;32(9):4022–8.

116. Sugimoto K, Moriyasu F, Tsuchiya T, Nagakawa Y, Hosokawa Y, Saito K, et al. Irreversible Electroporation for Nonthermal Tumor Ablation in Patients with Locally Advanced Pancreatic Cancer: Initial Clinical Experience in Japan. Intern Med. 2018 Nov 15;57(22):3225–31.

117. Arcidiacono PG, Carrara S, Reni M, Petrone MC, Cappio S, Balzano G, et al. Feasibility and safety of EUS-guided cryothermal ablation in patients with locally advanced pancreatic cancer. Gastrointest Endosc. 2012 Dec 1;76(6):1142–51.

118. Belfiore MP, Ronza FM, Romano F, Ianniello G Pietro, De Lucia G, Gallo C, et al. Percutaneous CT-guided irreversible electroporation followed by chemotherapy as a novel neoadjuvant protocol in locally advanced pancreatic cancer: Our preliminary experience. Int J Surg. 2015 Sep 1;21(S1):S34–9.

119. Akinwande O, Ahmad SS, Van Meter T, Schulz B, Martin RCG. CT Findings of Patients Treated with Irreversible Electroporation for Locally Advanced Pancreatic Cancer. J Oncol. 2015 Nov 5;2015:1–8.

120. Frigerio I, Girelli R, Giardino A, Regi P, Salvia R, Bassi C. Short term chemotherapy followed by radiofrequency ablation in stage III pancreatic cancer: results from a single center. J Hepatobiliary Pancreat Sci. 2013 Aug 1;20(6):574–7.

121. Girelli R, Frigerio I, Giardino A, Regi P, Gobbo S, Malleo G, et al. Results of 100 pancreatic radiofrequency ablations in the context of a multimodal strategy for stage III ductal adenocarcinoma. Langenbeck's Arch Surg. 2013 Jan 29;398(1):63–9.

122. Probst P, Hüttner FJ, Meydan Ö, Abu Hilal M, Adham M, Barreto SG, et al. Evidence Map of Pancreatic Surgery–A living systematic review with meta-

analyses by the International Study Group of Pancreatic Surgery (ISGPS). Surgery. 2021 Nov 27;170(5):1517–24.

123. Macedo FIB, Jayanthi P, Mowzoon M, Yakoub D, Dudeja V, Merchant N. The Impact of Surgeon Volume on Outcomes After Pancreaticoduodenectomy: a Meta-analysis. J Gastrointest Surg. 2017 Oct 25;21(10):1723–31.

124. Ansari D, Kristoffersson S, Andersson R, Bergenfeldt M. The role of irreversible electroporation (IRE) for locally advanced pancreatic cancer: a systematic review of safety and efficacy. Scand J Gastroenterol. 2017 Nov 2;52(11):1165–71.

125. Philips P, Hays D, Martin RCG. Irreversible Electroporation Ablation (IRE) of Unresectable Soft Tissue Tumors: Learning Curve Evaluation in the First 150 Patients Treated. Zhang Z, editor. PLoS One. 2013 Nov 1;8(11):e76260.

126. O'Neill CH, Martin RCG. Cardiac synchronization and arrhythmia during irreversible electroporation. J Surg Oncol. 2020 Sep 1;122(3):407–11.

127. Deodhar A, Dickfeld T, Single GW, Hamilton WC, Thornton RH, Sofocleous CT, et al. Irreversible Electroporation Near the Heart: Ventricular Arrhythmias Can Be Prevented With ECG Synchronization. Am J Roentgenol. 2011 Mar;196(3):W330–5.

128. Jarm T, Krmac T, Magjarevic R, Kos B, Cindric H, Miklavcic D. Investigation of safety for electrochemotherapy and irreversible electroporation ablation therapies in patients with cardiac pacemakers. Biomed Eng Online. 2020 Dec 16;19(1):85.

129. Martin RC, Schwartz E, Adams J, Farah I, Derhake BM. Intra-operative Anesthesia Management in Patients Undergoing Surgical Irreversible Electroporation of the Pancreas, Liver, Kidney, and Retroperitoneal Tumors. Anesthesiol Pain Med. 2015 Apr 20;5(2):22786.

130. Månsson C, Nilsson A, Karlson B-M. Severe complications with irreversible electroporation of the pancreas in the presence of a metallic stent: a warning of a procedure that never should be performed. Acta Radiol Short Reports. 2014 Dec 1;3(11):204798161455640.

131. Månsson C, Nilsson A, Månsson D, Karlson B-M. Response from the authors of the original article. Comment to: Månsson C, Nilsson A, Karlson B-M. Severe complications with irreversible electroporation of the pancreas in the presence of a metallic stent: a warning of a procedure that never should be

per. Acta Radiol Open. 2015 Sep 1;4(9):205846011560387.

132. Scheffer HJ, Vogel JA, van den Bos W, Meijerink MR, Besselink MG, Verdaasdonk RM, et al. Comment to: Månsson C, Nilsson A, Karlson B-M. Severe complications with irreversible electroporation of the pancreas in the presence of a metallic stent: a warning of a procedure that never should be performed. Acta Radiologica Short Reports 2014;3(11):1. Acta Radiol Open. 2015 Jul 1;4(7):205846011558411.

133. Scheffer HJ, Vogel JA, van den Bos W, Neal RE, van Lienden KP, Besselink MGH, et al. The Influence of a Metal Stent on the Distribution of Thermal Energy during Irreversible Electroporation. Scarfi MR, editor. PLoS One. 2016 Feb 4;11(2):e0148457.

134. Hogenes AM, Slump CH, te Riet o.g. Scholten GA, Meijerink MR, Fütterer JJ, van Laarhoven CJHM, et al. Effect of irreversible electroporation parameters and the presence of a metal stent on the electric field line pattern. Sci Rep. 2020 Dec 11;10(1):13517.

135. Rohan T, Andrasina T, Juza T, Matkulcik P, Červinka D, Svobodova I, et al. Experimental model of occluded biliary metal stent recanalization using irreversible electroporation via a tubular catheter. Int J Hyperth. 2021 Jan 1;38(1):393–401.

136. Moris D, Machairas N, Tsilimigras DI, Prodromidou A, Ejaz A, Weiss M, et al. Systematic Review of Surgical and Percutaneous Irreversible Electroporation in the Treatment of Locally Advanced Pancreatic Cancer. Ann Surg Oncol. 2019 Jun 6;26(6):1657–68.

137. Holland MM, Bhutiani N, Kruse EJ, Weiss MJ, Christein JD, White RR, et al. A prospective, multi-institution assessment of irreversible electroporation for treatment of locally advanced pancreatic adenocarcinoma: initial outcomes from the AHPBA pancreatic registry. HPB. 2019 Aug;21(8):1024–31.

138. Ruarus AH, Vroomen LGPH, Geboers B, van Veldhuisen E, Puijk RS, Nieuwenhuizen S, et al. Percutaneous Irreversible Electroporation in Locally Advanced and Recurrent Pancreatic Cancer (PANFIRE-2): A Multicenter, Prospective, Single-Arm, Phase II Study. Radiology. 2020 Jan;294(1):212–20.

139. Vogel JA, Rombouts SJ, de Rooij T, van Delden OM, Dijkgraaf MG, van Gulik TM, et al. Induction Chemotherapy Followed by Resection or Irreversible Electroporation in Locally Advanced Pancreatic Cancer

(IMPALA): A Prospective Cohort Study. Ann Surg Oncol. 2017 Sep 30;24(9):2734–43.

140. Narayanan G, Bhatia S, Echenique A, Suthar R, Barbery K, Yrizarry J. Vessel Patency Post Irreversible Electroporation. Cardiovasc Intervent Radiol. 2014 Dec 12;37(6):1523–9.

141. Dunki-Jacobs EM, Philips P, Martin RC. Evaluation of Resistance as a Measure of Successful Tumor Ablation During Irreversible Electroporation of the Pancreas. J Am Coll Surg. 2014 Feb 1;218(2):179–87.

142. Flak RV, Malmberg MM, Stender MT, Hauberg A, Thorlacius-Ussing O. Irreversible electroporation of pancreatic cancer – Effect on quality of life and pain perception. Pancreatology. 2021 Sep;21(6):1059–63.

143. Field W, Rostas JW, Martin RCG. Quality of life assessment for patients undergoing irreversible electroporation (IRE) for treatment of locally advanced pancreatic cancer (LAPC). Am J Surg. 2019;218(3):571–8.

144. Litière S, Collette S, de Vries EGE, Seymour L, Bogaerts J. RECIST — learning from the past to build the future. Nat Rev Clin Oncol. 2017 Mar 20;14(3):187–92.

145. Ye C, Sadula A, Ren S, Guo X, Yuan M, Yuan C, et al. The prognostic value of CA19-9 response after neoadjuvant therapy in patients with pancreatic cancer: a systematic review and pooled analysis. Cancer Chemother Pharmacol. 2020 Dec 12;86(6):731–40.

146. Ballehaninna UK, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. J Gastrointest Oncol. 2012;3(2):105–19.

147. Katz MHG, Fleming JB, Bhosale P, Varadhachary G, Lee JE, Wolff R, et al. Response of borderline resectable pancreatic cancer to neoadjuvant therapy is not reflected by radiographic indicators. Cancer. 2012 Dec 1;118(23):5749–56.

148. He C, Wang J, Zhang Y, Lin X, Li S. Irreversible electroporation after induction chemotherapy versus chemotherapy alone for patients with locally advanced pancreatic cancer: A propensity score matching analysis. Pancreatology. 2020 Apr;20(3):477–84.

149. van Veldhuisen E, Vroomen LG, Ruarus AH, Derksen TC, Busch OR, de Jong MC, et al. Value of CT-Guided Percutaneous Irreversible

Electroporation Added to FOLFIRINOX Chemotherapy in Locally Advanced Pancreatic Cancer: A Post Hoc Comparison. J Vasc Interv Radiol. 2020 Oct;31(10):1600–8.

150. Ma Y-Y, Leng Y, Xing Y-L, Li H-M, Chen J-B, Niu L-Z. Gemcitabine plus concurrent irreversible electroporation vs gemcitabine alone for locally advanced pancreatic cancer. World J Clin Cases. 2020 Nov 26;8(22):5564–75.

151. Byun Y, Han Y, Kang JS, Choi YJ, Kim H, Kwon W, et al. Role of surgical resection in the era of FOLFIRINOX for advanced pancreatic cancer. J Hepatobiliary Pancreat Sci. 2019 Sep 24;26(9):416–25.

152. Bower M, Sherwood L, Li Y, Martin R. Irreversible electroporation of the pancreas: Definitive local therapy without systemic effects. J Surg Oncol. 2011 Jul 1;104(1):22–8.

153. Charpentier KP, Wolf F, Noble L, Winn B, Resnick M, Dupuy DE. Irreversible electroporation of the pancreas in swine: a pilot study. HPB. 2010 Jun;12(5):348–51.

154. Edhemovic I, Brecelj E, Cemazar M, Boc N, Trotovsek B, Djokic M, et al. Intraoperative electrochemotherapy of colorectal liver metastases: A prospective phase II study. Eur J Surg Oncol. 2020 Sep;46(9):1628–33.

155. Coletti L, Battaglia V, De Simone P, Turturici L, Bartolozzi C, Filipponi F. Safety and feasibility of electrochemotherapy in patients with unresectable colorectal liver metastases: A pilot study. Int J Surg. 2017 Aug 1;44:26–32.

156. Djokic M, Cemazar M, Popovic P, Kos B, Dezman R, Bosnjak M, et al. Electrochemotherapy as treatment option for hepatocellular carcinoma, a prospective pilot study. Eur J Surg Oncol. 2018 May 1;44(5):651–7.

157. Bhutiani N, Agle S, Li Y, Li S, Martin RCG. Irreversible electroporation enhances delivery of gemcitabine to pancreatic adenocarcinoma. J Surg Oncol. 2016 Aug 1;114(2):181–6.

158. Bhutiani N, Li Y, Zheng Q, Pandit H, Shi X, Chen Y, et al. Electrochemotherapy with Irreversible Electroporation and FOLFIRINOX Improves Survival in Murine Models of Pancreatic Adenocarcinoma. Ann Surg Oncol. 2020 Oct 17;27(11):4348–59.

159. Casadei R, Ricci C, Ingaldi C, Alberici L, Di Marco M, Guido A, et al. Intraoperative electrochemotherapy in locally advanced pancreatic cancer:

indications, techniques and results—a single-center experience. Updates Surg. 2020 Dec 12;72(4):1089–96.

160. Petersen LJ, Zacho HD. PSMA PET for primary lymph node staging of intermediate and high-risk prostate cancer: an expedited systematic review. Cancer Imaging. 2020 Dec 23;20(1):10.

161. Neal RE, Rossmeisl JH, Robertson JL, Arena CB, Davis EM, Singh RN, et al. Improved Local and Systemic Anti-Tumor Efficacy for Irreversible Electroporation in Immunocompetent versus Immunodeficient Mice. Rubinsky B, editor. PLoS One. 2013 May 24;8(5):e64559.

162. Zhao J, Wen X, Tian L, Li T, Xu C, Wen X, et al. Irreversible electroporation reverses resistance to immune checkpoint blockade in pancreatic cancer. Nat Commun. 2019 Dec 22;10(1):899.

163. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, et al. Phase 2 Trial of Single Agent Ipilimumab (Anti-CTLA-4) for Locally Advanced or Metastatic Pancreatic Adenocarcinoma. J Immunother. 2010 Oct;33(8):828–33.

164. Le DT, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, et al. Evaluation of Ipilimumab in Combination With Allogeneic Pancreatic Tumor Cells Transfected With a GM-CSF Gene in Previously Treated Pancreatic Cancer. J Immunother. 2013 Sep;36(7):382–9.

165. Aglietta M, Barone C, Sawyer MB, Moore MJ, Miller WH, Bagalà C, et al. A phase I dose escalation trial of tremelimumab (CP-675,206) in combination with gemcitabine in chemotherapy-naive patients with metastatic pancreatic cancer. Ann Oncol. 2014 Sep;25(9):1750–5.

166. Brahmer JR, Tykodi SS, Chow LQM, Hwu W-J, Topalian SL, Hwu P, et al. Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer. N Engl J Med. 2012 Jun 28;366(26):2455–65.

167. Andersson R, Pereira C, Bauden M, Ansari D. Is immunotherapy the holy grail for pancreatic cancer? Immunotherapy. 2019 Dec;11(17):1435–8.

168. Zhang Q-W, Guo X-X, Zhou Y, Wang Q-B, Liu Q, Wu Z-Y, et al. OX40 agonist combined with irreversible electroporation synergistically eradicates established tumors and drives systemic antitumor immune response in a syngeneic pancreatic cancer model. Am J Cancer Res. 2021;11(6):2782–801.

169. Go E-J, Yang H, Chon HJ, Yang D, Ryu W, Kim D-H, et al. Combination of

Irreversible Electroporation and STING Agonist for Effective Cancer Immunotherapy. Cancers (Basel). 2020 Oct 26;12(11):3123.

170. Narayanan JSS, Ray P, Hayashi T, Whisenant TC, Vicente D, Carson DA, et al. Irreversible Electroporation Combined with Checkpoint Blockade and TLR7 Stimulation Induces Antitumor Immunity in a Murine Pancreatic Cancer Model. Cancer Immunol Res. 2019 Oct;7(10):1714–26.

171. Brock RM, Beitel-White N, Davalos R V., Allen IC. Starting a Fire Without Flame: The Induction of Cell Death and Inflammation in Electroporation-Based Tumor Ablation Strategies. Front Oncol. 2020 Jul 28;10:1235.

172. He C, Huang X, Zhang Y, Lin X, Li S. T-cell activation and immune memory enhancement induced by irreversible electroporation in pancreatic cancer. Clin Transl Med. 2020 Jun 4;10(2).

173. Shao Q, O'Flanagan S, Lam T, Roy P, Pelaez F, Burbach BJ, et al. Engineering T cell response to cancer antigens by choice of focal therapeutic conditions. Int J Hyperth. 2019 Jan 1;36(1):130–8.

174. O'Neill C, Hayat T, Hamm J, Healey M, Zheng Q, Li Y, et al. A phase 1b trial of concurrent immunotherapy and irreversible electroporation in the treatment of locally advanced pancreatic adenocarcinoma. Surgery. 2020 Oct;168(4):610–6.

175. He C, Sun S, Zhang Y, Li S. Irreversible Electroporation Plus Anti-PD-1 Antibody versus Irreversible Electroporation Alone for Patients with Locally Advanced Pancreatic Cancer. J Inflamm Res. 2021 Sep;Volume 14:4795–807.

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