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Irreversible electroporation for the management of pancreatic cancer. Current data and future directions

Spiliopoulos S *et al*. IRE for pancreatic cancer

Abstract

Pancreatic cancer is currently the seventh leading cause of cancer death (4.5% of all cancer deaths) while 80%-90% of the patients suffer from unresectable disease at the time of diagnosis. Prognosis remains poor, with a mean survival up to 15 mo following systemic chemotherapy. Loco-regional thermal ablative techniques are rarely implemented due to the increased risk of thermal injury to the adjacent structures. leading to severe adverse events. Irreversible electroporation (IRE), a novel promising, non-thermal, ablative modality, has been recently introduced in clinical practice for the management of inoperable pancreatic cancer, as a safer and more effective loco-regional treatment option. Experimental and initial clinical data are optimistic. The review will focus on the basic principles of IRE technology, currently available data, as well as future directions.

Key Words: Pancreatic cancer; Interventional oncology; Irreversible electroporation; Ablation; Loco-regional treatment; Image-guided treatment

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Core Tip: Loco-regional thermal ablative techniques such as radiofrequency, microwave and cryoablation are rarely implemented for the treatment of inoperable pancreatic cancer, due to the increased risk of thermal injury to the adjacent structures. Irreversible electroporation is a novel promising, non-thermal, ablative modality, that could provide a safer and effective ablation, *via* the application of electric pulses to damage cell membrane and cell homeostasis resulting in both cancer cell necrosis and apoptosis. Experimental and initial clinical data are optimistic, while its potential immunomodulatory effect, and synergistic therapy in combination with immunotherapy provides an optimistic prospective.

INTRODUCTION

Pancreatic cancer is currently the seventh leading cause of cancer death, representing 4.5% of all cancer deaths worldwide. Most importantly, overall prognosis remains extremely poor as approximately 80%-90% of the patients suffer from unresectable disease at the time of diagnosis, with a less than 8% relative survival rate at 5-years[1]. Systemic chemotherapy using gemcitabine or more recently FOLFORINOX regiments, with or without radiotherapy, result in overall survival rates ranging from 9 to 14 mo[2,3]. Moreover, thermal (radiofrequency and microwave) and cryo-ablative techniques are not commonly employed due to the increased risk of severe trauma to the adjacent major anatomical structures[4]. Irreversible electroporation (IRE) is a novel promising percutaneous, image-guided, nonthermal, ablative modality that has been recently introduced in clinical practice for the management of pancreatic cancer.

Mechanism of action

The phenomenon of IRE has been first reported in the 70's to describe the alteration of transmembrane potential leading to the increased cell membrane permeability, disruption of the dual lipid layer and the creation of permanent nanoscale defects (nanopores) in the cell membrane, following the application of high voltage, pulsed electric fields, across the cell[5]. This results in failure of the cell homeostasis, electrolyte alteration and cell death by apoptosis[6-9].

In contrast to necrosis induced by thermal ablative methods, non-thermal, apoptotic active cell death does not incite inflammation and enables ablation with minimal distortion of the adjacent tissues. However, since 2006, when the first *in vivo* model of IRE for cancer ablation was reported, several experimental studies reported solely necrosis or mixed results of both necrosis and apoptosis following the application of IRE[10-13]. According to currently available experimental data apoptosis has been demonstrated immediately after IRE in a murine cancerous pancreas model and between 7 and 14 d in a porcine healthy pancreas animal model, while necrosis is

evident immediately and up to 14 d. Unfortunately, pathology data on human pancreatic cancer are extremely limited and as the IRE ablative effect is directly correlated to the physical properties of the target tissue, the significant discrepancies between *in vivo*, normal/cancer animal models and human cancer/normal pancreatic tissues present a major limitation regarding our actual knowledge on the real effect of IRE[14-16]. Moreover, data indicate that IRE is not homogenously distributed along the target tissue and various effects are produced with increasing voltage and time.

While apoptosis is certainly occurring in some cells within the treatment zone, Brock *et al*[17] suggest that IRE could initiate multiple types of cell death mechanisms, but the size and shape of the regions in which each type is experienced may vary between clinical treatments depending on differences in pulsing parameters, tissue type, and treatment time. Thus, there may be more than one type of cell death mechanism at play, including pyroptosis and necroptosis. Likewise, for cells at the margins of the treatment areas, the response may actually be survival signaling to reversible electroporation. In theory, this could be taken advantage of and combined with chemotherapy treatments to increase drug delivery, tumor penetration, and treatment of remnant cancer cells[17,18].

IRE has significant inherent advantages over thermal ablation for the treatment of pancreatic cancer. Most importantly IRE does not produce temperature increase to achieve tumor destruction, therefore it does not elicit thermal injury to the superior mesenteric and portal veins, superior mesenteric and celiac arteries, bile duct adjacent nerves, and gastrointestinal structures, which has restrained the use of local thermal ablation treatment. Another significant advantage is the absence of the "heat sink" effect in which the flow of large blood vessels decreases the thermal ablative effect[19-22]. According to published clinical protocols, the procedure is performed under computed tomography (CT)-guidance, general anesthesia (complete muscle paralysis) and electrocardiography synchronization, due to the possibility of muscular spasms induced by high-voltage, pulses[23]. IRE is induced by electrodes (needles), connected to a high-voltage pulse generator. Multiple electrode pairs can be used. The number of needles needed and their exact placement, is decided during pre-procedural planning.

For small tumors measuring up to 2 cm three electrodes are placed at the periphery of the target lesion, and for lesions between 2 and 3 cm four electrodes are used. However, for lesions > 3 cm a maximum number of six needled is allowed with four or five electrodes at the periphery and one or two at the center of the lesion. An optimal interelectrode distance between 7 and 24 mm has been described.

The correctly positioning of electrodes requires skills and experience often deriving from other ablative methods. It is also necessary to acquire skills in using ultrasound or CT support as a guide for positioning the needles and avoiding accidentally damage in surrounding organs.

Regarding large vessels close to the tumor, a minimum safety distance of 2 mm is recommended to avoid the risk of burn damage. In cases of locally advanced pancreatic cancer with involvement of the mesenteric artery/vein, placing the needles parallel to the vessels has been proven effective. Following needle placement, the generator produces short, repeated, pulses, using predetermined voltage settings, to reach a target current of 20-50 A. In clinical practice usually, 90 pulses per treatment cycle are used, with a pulse duration of 70-90 μs, and a voltage setting between 1400-1800 V/cm (maximum capability 3000 V/cm)[2,24-26]. According to standard ablation technique, the aim is to create a safe 5 mm tumor-free, IRE zone, also referred as A0 ablation on analogy to a R0 surgical resection.

Pre-clinical data

To date, only few reports on IRE for treatment of pancreatic cancer exist in literature. Some of them reported the experience on animal models (Table 1). These research studies have used animal xenografts carrying human pancreatic cancer cells to understand the histological effect of IRE on pancreatic cancer tissue. These studies implanted the human pancreas cancerous cell lines in the animal models. The results after IRE showed the evidence of both acute coagulative necrosis and apoptosis of pancreatic cancer tissue followed by fibrosis.

In 2010, Charpentier *et al*[27] reported a pilot study on IRE in a normal pancreas porcine model. They showed the following histological features: An initially induced active local inflammation, with oedema of the interstitium and after 7 d reported a significant necrosis followed by the development of fibrosis. However, these results were not significant for IRE efficacy because normal pancreatic tissues had a very different conductivity than pancreatic tumors.

Subsequently, Bower *et al*[28] and José *et al*[29] suggested that IRE could be an effective treatment for locally advanced pancreatic cancer in a mouse model without systemic toxicity and major local complication. Su *et al*[30] concluded that IRE was a safer and shorter operating procedure than traditional ablative techniques and represented a promising new approach for pancreatic cancer. Narayanan *et al*[31] described IRE for pancreatic cancer mouse model concluding that animal model serves as a robust system to study the effects and clinical efficacy of IRE. Also Lee *et al*[32] demonstrated and confirmed on porcine model the safety and minimal complications of IRE ablation in the pancreatic cancer tissue. The results of IRE in animals model for the pancreatic cancer treatment showed the ability to ablate the pancreas cells preserving the collagen architecture of vascular, biliary, or neuronal structures[28,33].

Clinical data

The prognosis of patients with pancreatic cancer not eligible for surgery remains poor despite many chemoradiation protocols, therefore different approaches are required. Ablation procedure including radiofrequency ablation, microwave ablation, cryo-ablation, high intensity focused ultrasound and IRE can offer symptomatic relief, survival benefit and potential downsizing. Nevertheless thermal procedures, with extreme temperature, induce injury to the pancreatic duct, bile duct and to adjacent vessels that can result in fistulae, bile leaks and bleeding respectively[34].

IRE is an emerging, safe and non-thermal local ablation technique that affects with electricity only target cell membranes and saves the vessels and vital structure. Therefore, IRE can also be used in tumors positioned near some vital structures or

organs compared to other ablative procedure[35]. The main current indication of IRE for pancreatic cancer are the following: Locally advanced pancreatic cancer stage II or III (T4N1M0) with regional positive lymph nodes ≤ 3, According to the American Joint Committee on Cancer stage criteria (8th edition); tumor size maximal axial diameter ≤ 5 cm; patients not candidates for radical resection or someone who refuses the surgical operation. IRE has also some absolute and relative contraindications: It cannot be used if there are metal implants less than 2.5 cm from the ablation area, patients with portal vein occlusion, portal hypertension, ascites, bile duct obstruction and hyperbilirubinemia. IRE work on myocardial contraction mechanisms and it cannot be applied in case of cardiac arrhythmias, previous heart failure, active coronary disease and peacemakers implanted within 1 year. IRE cannot be used in epilepsy even if it has not been proven to cause brain stimulation[35].

The indication and contraindications of IRE for pancreatic cancer are summarized on Table 2. Martin *et al*[36] and Narayanan *et al*[37] described the first clinical series on IRE treatment for human pancreatic cancer. Since then, the use of this technique has been widespread and other studies have been published[38-40], but to date there is still no defined protocol for IRE in pancreatic cancer. Studies showed that IRE was a treatment for locally advanced pancreatic cancer or borderline resectable pancreatic cancer because allowed tumor downstaging, definitive locoregional treatment or adjuvant treatment to resection[41-43]. In human tumour tissue IRE induces necrosis as in animal cancer models, however there is no evidence of apoptosis[16]. A series of retrospective and prospective clinical studies on human pancreatic cancer treated with IRE suggested a survival benefit with a median overall survival (OS) up to 30 mo[38,39].

Combined treatments involving IRE, chemotherapy and immunotherapy can offer a multimodal approach which limits the progression of the disease. The debate is open on the timing of multimodal treatment: Some studies that administered IRE before chemotherapy showed only a modest increase in survival, Månsson *et al*[40] reported a median OS of 13 mo, instead studies using IRE after induction chemotherapy reported an increase in survival with a median OS rate of 27 mo[44]. Despite improvements in

radiation therapy, chemotherapy and surgical procedures over the last 30 years, pancreatic cancer 5-year survival rate remains at 9%.

Recently, the advanced technique of proton radiation end Carbon ion radiation therapy has been used for locally advanced pancreatic cancer, producing encouraging results. The proton beam offers significant physical advantages over the photon due to the Bragg peak effect with little or no output dose beyond the tumor target, thereby sparing the critical organs adjacent to cancer. Compared to proton, carbon ion offers similar dosimetric characteristics, but it has a substantially different biological property. Carbon ions have greater biological efficacy inducing more complex DNA damages and leading to an increase cancer cells killing[44].

Despite the non-thermal effect of IRE, the production of heat near the electrodes it remains unavoidable and was defined as secondary Joule heating[45]. Serious complications after IRE related to the location and size of pancreatic cancer have been reported in the literature; the risks depend on the proximity to vital structures such as large blood vessels and main biliary and pancreatic duct. The most common complications are mild acute pancreatitis, pain, diarrhoea, nausea, vomiting, loss of appetite and delayed gastric emptying but there are also serious complications such as arrhythmia, severe acute pancreatitis, haemorrhage, portal vein thrombosis, bile or pancreatic fistula, gastro-intestinal tract perforations and death[16,45]. In one of the most recent reviews[16], the average serious complication rate after IRE was 12%, with a maximum reported value of 42%[2]. The size of the tumor is one of the most important factors related to procedure complications; for example Narayanan *et al*[46] treated patients with tumors up to 8 cm in size and reported one of the highest total complication rates of 62%. Instead, the average mortality rate reported was 2% for open IRE and 0% for percutaneous IRE[20]. Other factors contributing to post IRE complications depend on the team experience, the protocol used and the type of approach (open *vs* percutaneous)[18].

Protocols in part derive from animal studies data, however the pancreas of animals and humans are significantly different in term of cellular composition and electrical

impedance. Another problem arises from the treatment protocols of IRE present to date, which differ for distance between the electrodes, the intensity of the applied voltage and the individual electrical properties of the tissue being ablated. These variabilities have an impact on the effectiveness of the treatment and on the area of ablation[16].

This is an important knowledge gap due to the risks and ethics of *in vivo* human tissue sampling. One way to obtain this information and solve this knowledge gap is to apply IRE to perfused human organs (both carcinogenic and healthy). Use IRE on *ex vivo* perfused pancreas, could define the development of a treatment protocol with IRE[16]. Actually, IRE is not yet in clinical practice because there isn't consensus on the optimal IRE treatment protocol and for the approach required to protect adjacent pancreatic tissue[25]. Evaluation of benefits following IRE are needed in pancreatic tumor tissue in order to establish appropriate treatment protocols.

Future directions

The main issue of IRE for pancreatic cancer is the absence of randomized data. Currently, two major randomized controlled trials (RCT) are recruiting patients in order to provide level 1 safety and efficacy evidence *vs* standard of care. The PAL-PIE is a United Kingdom-based multicenter RCT that will recruit 50 patients (up to seven pancreas centres) with locally advanced pancreatic cancer and previous first-line chemotherapy (5-fluorouracil, leucovorin, irinotecan and oxaliplatin) randomized to IRE (plus chemotherapy if indicated) *vs* chemotherapy alone[47]. The DIRECT is a United States-based trial (A Randomized, Multicenter, Controlled, Unblinded Study to Assess the Safety and Efficacy of the NanoKnife® System for the Ablation of Unresectable Stage 3 Pancreatic Adenocarcinoma) will randomize over 500 patients with stage III pancreatic cancer, to receive IRE plus chemotherapy (a modified FOLFIRINOX regimen) *vs* chemotherapy alone and the estimated completion date will be December 2023 (ClinicalTrials.gov Identifier: NCT03899636).

Additionally, following positive initial clinical outcomes researchers are currently investigating the very interesting concept of IRE-induced immune response to cancer[48-

[52]. IRE has been identified as a potential immunomodulatory therapy due to post-ablation significant release of intracellular tumoral antigens that act as *in situ* immunization agents resulting in both local and systemic response to remaining cancer cells. Specifically, IRE can remodel the local tumor microenvironment, by smoothening the extracellular matrix, alleviating hypoxia, and assisting the infiltration of immune cells into residual cancer. Moreover, the combination of IRE and immunotherapy could incite potent synergistic antitumoral effects[24]. Nevertheless, the mechanism causing immunomodulation following IRE in humans remains unknown[53]. However, trials focusing on the potential of IRE combined with immunotherapy to improve prognosis of unresectable pancreatic cancer are ongoing. For example, a pilot, multicenter, single-arm phase II trial is currently recruiting patients with metastatic pancreatic ductal adenocarcinoma to investigate whether the combination of IRE of one liver metastasis followed by nivolumab treatment leads to a measurable radiological response in a selected non-treated liver metastasis (IRE) (Followed by Nivolumab in Patients With Metastatic Pancreatic Cancer: a Multicenter Single-arm Phase II Trial; ClinicalTrials.gov Identifier: NCT04212026).

Combination therapy of IRE with chemotherapeutic regiments are also being evaluated as the rim of peripheral sensitivity to chemotherapy produced around central tumor necrosis following IRE, is a perfect target for pancreatic cancer which typically presents microscopic peripheral seeding[54]. In that direction results from several ongoing prospective trials such as the CHEMOFIRE-2 trial (Chemotherapy Followed by Irreversible Electroporation in Patients With Unresectable Locally Advanced Pancreatic Cancer; ClinicalTrials.gov Identifier: NCT04093141) are awaited.

Improvements regarding IRE technology are also awaited. Another interesting approach that requires further investigation is endoscopic IRE, that could provide a solution to patients without safe transabdominal access. A major limitation of IRE is the possibility to produce a small ablation zone of approximately 1-1.5 cm, and therefore several electrodes are required to create the desired A0 ablation, rending the procedure more technical demanding and time consuming compared to thermal ablation

modalities[55]. Future research should focus on the standardization and optimization of the treatment protocol in order to provide the maximum efficacy without damaging normal surrounding tissues, as well as post-treatment radiological assessment aiming in discovering objective and measurable predictors of outcomes.

CONCLUSION

As demonstrated by initial pre-clinical and clinical data, the unique characteristics of IRE render this non-thermal ablation modality most suitable for the minimal invasive treatment of locally advanced pancreatic cancer. The synergic effect of IRE combined with chemo- or immune-therapy could significantly improve outcomes. Further investigation is required to elucidate its exact mechanism of action, optimize the treatment protocol and provide high-quality comparative clinical data for the management of patients with pancreatic cancer.

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