Gastrointest Interv 2014; 3:8–18

Contents lists available at ScienceDirect

Gastrointestinal Intervention

journal homepage: www.gi-intervention.org

Review Article

Irreversible electroporation (NanoKnife) in cancer treatment

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A B S T R A C T

Irreversible electroporation (IRE) is a promising new minimally invasive modality for the ablation of solid tumors. Unlike the current leading thermal ablation modalities, such as radiofrequency ablation (RFA) and cryoablation, IRE uses nonthermal electric energy to irreversibly destabilize cell membranes, resulting in focused cell death. Over the past 7 years, IRE has been emerging as a novel ablation tool by using the effect of an applied electric field to kill cancer cells, without damaging the surrounding extracellular matrix, vessels, nerves, and neighboring normal tissue. Although IRE has been investigated for a short period of time, its potential use for cancer and tissue ablation has been receiving growing attention leading to a considerable number of studies on its validity and safety, including recent *in vivo* animal and human studies.

Keywords: Irreversible electroporation, Nonthermal ablation, Review, Tumor ablation

Introduction

Irreversible electroporation (IRE) is a novel nonthermal ablation modality with promise for revolutionizing the treatment for local solid tumors.[1–5] With the growing demand for alternative and less invasive treatments for localized tumors, we have seen the development and investigation of several tissue ablation modalities, including radiofrequency ablation (RFA), microwave ablation, and cryoablation. Although these modalities have been efficacious, they have some disadvantages owing to their reliance on thermal energy for creating cell death.[1,4] IRE is novel in that it does not use thermal energy, but electrical energy to produce focused cell death while sparing the normal extracellular matrix, nearby vessels, and structures, while allowing for rapid normal tissue regrowth.[1–10] Unlike thermal ablation modalities, IRE does not require significant consideration for dissipation of thermal energy, or heat sink, and has less complications relating to damage of normal soft tissue, eliminating a major cause of treatment failure.[2,3,5,10,11] Additionally, IRE treatment time is significantly shorter than traditional thermal ablation modalities, in low minute ranges, and may allow for treatment of considerably larger lesions than thermal ablation modalities.[7,12]

Although IRE has been investigated and utilized for only the past 7 years, its potential use for cancer ablation has been receiving growing attention leading to a considerable number of studies on its safety and efficacy. IRE has demonstrated effective cell death in normal tissue, cancer cell cultures, *in vivo* animal studies, and human clinical studies.[13–27] IRE is now being studied with great enthusiasm in several organ systems with promising data.

History

Electroporation is a technique in which strong electrical fields are used to create nano-sized pores in a cell's membrane, permeabilizing the cell membrane, and disrupting intracellular homeostasis.[12] The formation of pores by electroporation can be reversible or irreversible. Although reported as early as the 1750s, electroporation has been an important tool in medicine and research for only the past 30 years.[12] Reversible electroporation has been utilized in many medical applications, including electrogenotherapy to deliver genes into cells for gene therapy and also electrochemotherapy to deliver chemotherapeutic drugs into cells as an alternative method of treating solid tumors.[12,28–34] However, if the applied electric voltage is above a certain threshold, it leads to a larger potential gradient and the cells are unable to seal the formed pores and the result is cell death.[35]

Until 7 years ago, IRE was merely considered an undesirable side-effect of reversible electroporation as investigators were attempting to treat and cure cells and causing cell death would have been considered a failed attempt. Until recently, irreversible disruption of cell-membrane integrity by IRE had only found a practical use in microbial inactivation in the food industry.[36–39] Rubinsky's group[2,3,5] however, introduced the use of IRE as a method to deliberately induce irreversible disruption of cell membrane integrity in order to cause cell death. Over the past 5 years IRE has been emerging as a significant medical tool in its own right by using the effect of an applied electric field to kill cancer cells, as well as other undesirable tissue. IRE has demonstrated

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Received 23 September 2013; Revised 24 February 2014; Accepted 27 February 2014

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http://dx.doi.org/10.1016/j.gii.2014.02.002

effective cell death in normal tissue[2] and cancer cell cultures,[40] with the first *in vivo* use of IRE in animal studies reported by Edd et al[3] in 2006, and the first *in vivo* use reported in humans by Pech et al[20] in 2010.

The science behind IRE

IRE takes advantage of the electric potential gradient that exists across cell membranes to create permanent pores in the cell membrane. The IRE generator sends electric energy pulses that alter the cell's transmembrane potential, causing disruption of the lipid bilayer, and creating permanent nano-sized pores that irreversibly increase the permeability of the cell membrane .[2,3,5,12,41,42] The result is focused apoptotic cell death within seconds as the cell loses its ability to maintain homeostasis, and the creation of a well-demarcated region of ablation, with a sharp boundary between the treated and untreated areas.[3,7]

Molecular surgery

Recently, Lee et al[43] demonstrated nanometer-sized pores created by IRE using scanning electron microscopy. For the first time, they showed various sizes of nanopores formed on swine and rabbit liver cell membrane (Fig. 1). Esser et al[41,42] also shed light on the cellular scale phenomena, describing the electrical fields and the dynamics of pore formation during IRE. He used a multicellular system model composed of irregularly shaped liver cells at a 100-μm spatial scale and a multiscale liver tissue model at a 200-mm spatial scale.[41,42] Golberg et al[44] compared IRE with "molecular surgery" because only the cell membrane in the treated area is affected while other molecular structures in the tissue are spared.[12,45]

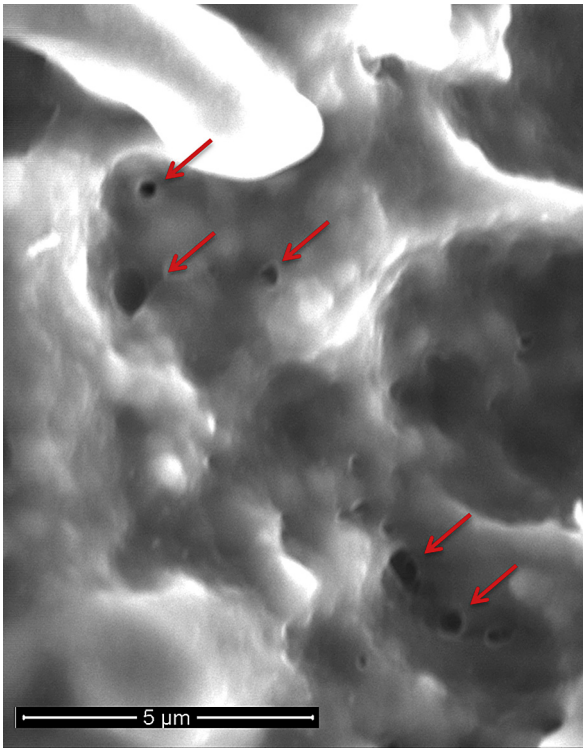

Fig. 1. Scanning electron microscopy image demonstrating multiple nano-sized pores (arrows) on the cell membrane of swine liver after ablated with IRE.

Tissue ablation by IRE versus thermal ablation modalities (apoptosis vs. necrosis)

Tissue ablation by thermal ablation modalities creates central necrosis, surrounded by a gradually increasing border of cell death with time as heat or cold dissipates at the peripheral border of the intended ablation zone.[1,5,46,47] Unlike these thermal ablation techniques, IRE causes complete cell death without the use of significant thermal energy, and without the associated inflammation and immunoreaction.[48]

Lee et al[6,7] were the first to demonstrate that cell death created by IRE has increased positive BAX (BCL-2 oncoprotein) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay staining compared with normal adjacent tissue, indicating the role of apoptosis in cell death created by IRE (Fig. 2). This is in contrast to the thermal ablation modalities where RFA creates coagulative necrosis, and cryoablation creates cell membrane rupture, leading to central necrosis of the ablated region.[1,4,12] Utilizing immortal human cervical adenocarcinoma HeLa cells, Zhou et al[48] were again recently able to demonstrate the principle behind IRE-induced apoptosis, showing that low-voltage IRE with more pulses induced the tumor cells to undergo apoptosis and effectively eliminate the HeLa cells. The significance is that the application of these short strong electrical pulses with IRE has little effect on the temperature of the treated tissue and the Joule heating induced temperature elevation in tissue by IRE reaches levels that are not harmful to surrounding tissues.[2,49]

Additionally, Lee et al[7] showed hepatocellular regeneration as early as 24 hours after IRE ablation, with another study by Rubinsky et al[10] demonstrating marked cellular tissue repair seen 14 days after IRE. These findings further support the role of IRE induced apoptotic cell death as apoptotic cells are promptly removed by immune cell derived phagocytosis and replaced by innate cellular regeneration.[7,10] This is in contrast to coagulative necrosis and protein denaturation induced by thermal ablation modalities, where necrotic cell death does not get replaced by intrinsic cellular regeneration but rather by fibrosis and scarring of cellular and tissue remnants. It is noteworthy that although immune cells play a role in the rapid resolution and healing of ablation with IRE, Al-Sakere et al[50] found that treatment success is not dependent on the immune system, although a tumor-specific immune response may be invoked.[9,50,51]

IRE spares the surrounding extracellular tissue

IRE's largely apoptotic effect lends to its promising clinical features, including the ability to affect specific cells, while sparing the surrounding extracellular tissue structure, and leaving blood vessels, bile ducts, the urethra, and nerves intact to function normally.[6–10,16,52–56] We believe that these structures are left fairly unharmed, because their higher collagenous connective tissue and elastic fiber contents lack a normal cellular membrane where IRE can create pores. This hypothesis is supported by evidence of mild vasculitis found in vessels within the IRE ablation zone, likely due to damage of endothelial cells lining the vessels, which lack collagenous and elastic fibrous tissues.[6] Another explanation that may explain the sparing of these structures is that gap junctions found within the cellular structure of these structures allow the electric currents of IRE to travel through the gap junctions from cell to cell without affecting the integrity of the cell membrane or surrounding connective tissue structures.[6] Specific to neural tissue, Schoellnast et al[53,57] demonstrated that nerves exposed to IRE were shown to maintain intact endoneurium and perineurium architecture, with Schwann cell proliferation, in contrast to nerves exposed to RFA which demonstrate coagulative necrosis. These

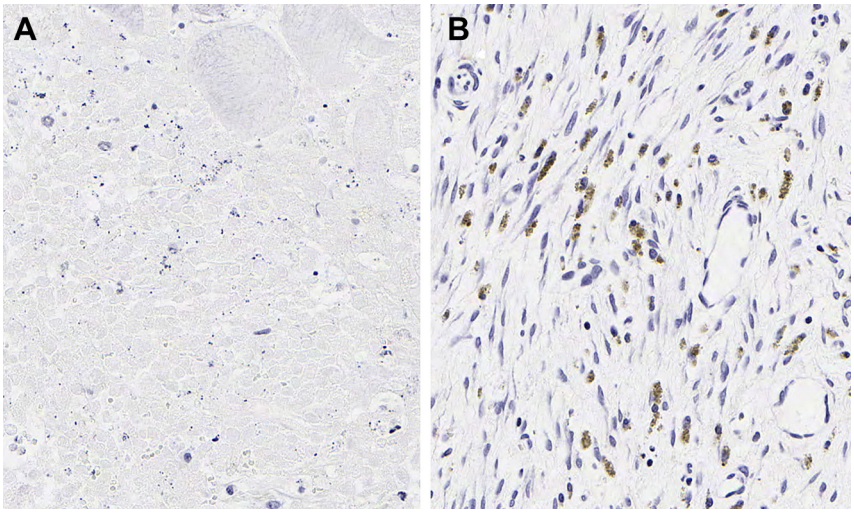

Fig. 2. Cell death by necrosis versus apoptosis. (A) Liver necrosis caused by radiofrequency ablation (RFA). (B) Apoptotic cell death caused by irreversible electroporation (IRE) – dark brown stains are positive for TUNEL assay. TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

findings suggest that nerves treated with IRE retain a potential for regeneration that is not present in nerves exposed to RFA.[53,57]

IRE is not affected by heat sink

Because IRE does not depend on heating for its ablative effects, it is not affected by heat sink, which is the cooling effect from blood flow that often hinders the full effect of thermal ablation modalities such as RFA for targeting ablation of hypervascular tumors or tumors near large vasculature.[4,52] This translates to the potential ability to completely ablate tumors up to the vessels without losing heat or cold via blood flow, as seen in RFA and cryoablation, respectively. This also means that IRE can ablate peri-vascular tumors such as a large hepatocellular carcinoma (HCC) around the portal vein or hepatic vein, which were considered nonablatable by RFA in the past.[7–10]

The IRE system and treatment protocol

IRE uses a direct current (DC) generator that creates short pulses of high voltage electric current to create irreversible pores in the cell membrane, thus, inducing apoptotic cell death in a target tissue.[9] The procedure requires a medical device specifically designed for clinical use and able to balance the delivery of high energy pulses with patient and operator safety. In 2007, Bertacchini et al[35] reported on the first IRE system approved for clinical use. Safety is an especially important concern with IRE because irreversibly electroporating a target volume of 50 cm^3 to 70 cm^3, requires generation of pulses of up to 3000 V of amplitude and currents up to 50 A.[35] The device must produce an electrical field gradient in a volume 40 cm^3 that is at least 800 V/cm, which was determined by Davalos et al[2] to be the threshold for IRE in most cell types.

The IRE system (Fig. 3, NanoKnife; AngioDynamics, Queensbury, NY,USA) consists of two main components: an IRE generator and up to six electrode probes. The generator can deliver between 100 V and 3000 V of energy in 90–100 pulses, with a maximum pulse length of 100 ms. The electrode probes are generally 15 cm in length and 16–19 gauge in diameter, and are inserted in or around the area of interest to be ablated. Two or more monopolar probes or a single bipolar probe must be used at a time, and an electrical field is created between them in a series of microsecond pulses inducing cell death. *In vitro* porcine data have shown that the bipolar probe is capable of creating an ellipsoid ablation zone with axes of approximately 30 mm and 15 mm.[20,35] The number of probes that are used during an IRE procedure is dependent on the size and shape of the desired zone of tissue ablation. Also, because the amplitude and the gradient of the field depend on the applied voltage as well as on the distance between the electrodes, the treatment parameter for voltage is dependent on the distance between probes within the targeted tissue.[20,35]

IRE treatment is minimally invasive, and can be performed with ultrasound (US) or computed tomography (CT) guidance. US, CT, or magnetic resonance imaging (MRI) may also be used post-procedurally to assess the extent of tissue ablation. The availability of pre- and especially peri-procedural imaging with US and CT, allows for accurate determination of tissue volume to be treated, and appropriate treatment planning and positioning of the electrodes.[6,7] An electrocardiogram (ECG) synchronizer should be used to synchronize IRE pulses with the refractory period of the cardiac rhythm in order to minimize the risk of arrhythmias.[14,20,35,58] The overall time for the IRE treatment procedure is extremely short, lasting minutes as ablation typically requires 90–100 pulses, corresponding to the same number of heart beats because IRE is coupled to the cardiac rate.[20,35]

Traditional IRE protocols achieve ablation through a series of unipolar electric pulses that result in significant muscle contractions. Because IRE treatment requires the patient to remain motionless, IRE is typically administered under general anesthesia with administration of neuromuscular blocking agents, such as atracurium or pancuronium, to prevent muscle contraction. Goldberg and Rubinsky[59] have proposed a technique to mitigate the volume of untargeted tissue that is vulnerable to muscle contraction during IRE. The authors suggest that surrounding a central energized electrode with a series of grounded electrodes reduces the volume of tissue exposed to electric fields with the potential to induce contraction. This novel approach, which is based on the Faraday cage concept, requires that at least 16 grounded electrodes surround one superficially inserted, energized electrode. Based on Goldberg and Rubinsky's[59] model, this configuration significantly reduces the volume of untargeted tissue that experiences contractions. Upon clinical translation, this innovation may reduce the amount of muscle relaxants that must be administered to achieve sufficient paralysis.[59]

Arena et al[60] recently developed a slightly modified approach, known as high-frequency irreversible electroporation (H-FIRE), that utilizes high frequency, bipolar bursts to eliminate muscle contraction, without sacrificing the efficiency of cell death due to

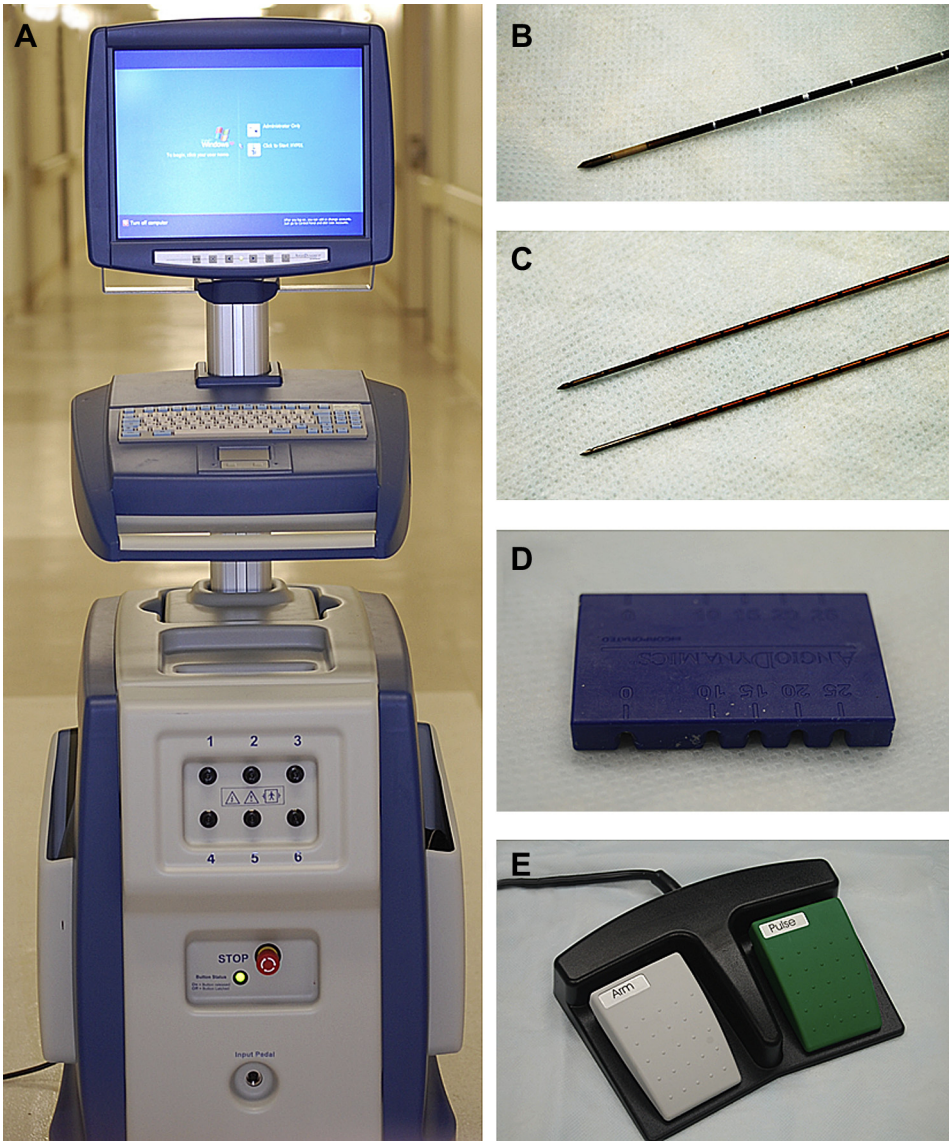

Fig. 3. Current IRE system. (A) IRE generator from AngioDynamics Inc. (B) 16G Bipolar IRE probe. (C) 19G monopolar IRE probes. (D) Monopolar IRE probe spacer. (E) IRE generator pedal. IRE, irreversible electroporation.

nonthermal electroporation. Rat brain tissue was subjected to 90 pulses of IRE, and H-FIRE at 250 kHz or 500 kHz. Although IRE led to characteristic muscle contractions, H-FIRE treatment at either frequency did not produce visual or tactile evidence of muscle contraction. Histopathological examination of the tissues revealed that H-FIRE at 250 kHz or 500 kHz was indistinguishable from IRE with respect to ablation efficacy and precision.[60] The clinical application of H-FIRE may eventually eliminate the need to administer neuromuscular agents during the IRE procedure.

Treatment protocol

Although the IRE procedure is relatively simple to perform, successful usage and outcome requires development of a treatment protocol based on a thorough understanding of how currents flow within various tissues. Treatment planning is crucial for IRE. Until recently, treatment planning to ensure complete ablation of the desired lesion consisted of mathematical formulations based on a deterministic model, using a deterministic single value for the amplitude of the electric field required for causing cell death.[45] This model assumed that IRE is associated with a single value of local electric field current and heat distribution during pulse application. However, as Golberg et al[44] explains, this is only correct when the cell population is homogeneous and uniform, which is not the case for tumor cells which are conversely heterogeneous and in different stages of development.

IRE outcome success depends on a number of parameters including the number, length, and shape of electric pulses, the interval between pulses, field amplitude, and polarity.[44] Especially important are the cell parameters of type, morphology, age, and size.[5,61–66] Past studies have described that the electric fields which occur in tissue ablation can be complex and variable, and that the treatment protocol must take into consideration the electric field distribution within the tissue which will significantly direct the effect of IRE, depending on parameters such as the electrode configuration, pulse parameters, and tissue heterogeneities .[2,45] The point is to use these parameters in order to ensure that the electric conditions created will destroy all the undesirable cells throughout the entire volume of target tissue, and leave the normal tissues we want to maintain unharmed. Thus Golberg et al[44] have

described a mathematical methodology for treatment planning that can take these parameters into consideration. It has long been known for example that in a population of aging cells, there is a statistical distribution, correlating cell survival to electroporation parameters.[67,68] Therefore, Golberg et al[44] use this model to adapt the previously employed deterministic model, into a novel statistical model of IRE-induced cell death to be used in making a more accurate type of treatment planning for more complete ablation of cancerous tumor cells in tissue.[44]

Ben-David et al[69] set out to determine the optimum parameters to perform precise liver ablation by IRE. US-guided IRE ablation was performed on 25 live porcine livers. The investigators systematically varied the number of pulses (20–90), length of pulses (20–100 ms), voltage (2250–3000 V), distance between electrodes (1.5–2.5 cm), and length of active electrode exposure (1.0–3.0 cm). The study concluded that all parameters were influential on IRE outcomes, and that accurate zones of ablation could be achieved by precise calibration of IRE protocols.[69] Also described by Ben-David et al[69] and based on our personal experience, the most challenging parameter to optimize is accurate electrode placement. Even with a laparotomy approach, an accurate electrode placement is difficult to achieve with current monopolar electrode (19 gauge) which is very flimsy and unstable when it is inserted. As the IRE ablation outcome is absolutely dependent on electrode distance and configuration within the tumor, a more innovative method of accurately placing the electrodes is needed. A new design of electrode, electrode stabilizer, and multi-imaging modality guidance have been implemented and currently active research projects are being conducted.

Safety considerations

Recently, Raffa et al[70] demonstrated that performing IRE in the presence of fibril boron nitride nanotubes (BNNTs) lowers the necessary voltage threshold required to cause tumor cell death. BNNTs consist of hexagonal arrangements of B and N atoms with the potential to amplify an electrical field within a cell. Comparing IRE in the absence and in the presence of BNNTs revealed that BNNTs significantly decrease the required voltage to produce cell death. Of note, IRE at 800 V/cm was 2.2 times more effective at causing cell destruction when performed in the presence of BNNTs.[70] These results suggest the potential use of BNNTs to increase the safety of IRE procedures by lowering the voltage threshold required to cause tumor cell death.

Need for ECG synchronization

Considerations of the IRE procedure and of appropriate patient selection include the need for use of ECG synchronization, muscle relaxant, and placement of several electrodes in cases of larger tumors.[14,20,58] The likelihood of an IRE treatment to cause arrhythmia depends on a number of factors including: the applied pulse voltage, pulse repetition rate, length and number of pulses, and the distance between the electrodes and the heart.[58,71] Therefore, synchronization of treatment delivery with the refractory period of the cardiac cycle is important, especially when there is not enough confidence that IRE cannot determine a current density lower than fibrillation threshold of the myocardium.[35,58]

In previous studies, IRE has been performed using parameters of 2000–3000 V at a high rate of 240 pulses per minute (PPM). du Pré et al[72] has recently demonstrated that IRE is unlikely to cause damage to coronary arteries, even when the treatment is targeted toward the coronary artery region.[72] Notwithstanding the absence of damage to critical cardiac structures, there are lingering safety concerns regarding the use of IRE's high electric energy in treating tumors located near the heart. These types of strong electric pulses may potentially cause arrhythmias such as ventricular flutter and fibrillation especially when the electrical pulses are delivered during the "vulnerable period" of the heart cycle or in patients with underlying arrhythmias.[58,73,74]

With the demand for an effective local ablative therapy for treating tumors located close to the heart, such as a perihilar mass or a hepatic tumor at the dome, Mali et al[73] developed an algorithm for the synchronization of IRE pulse delivery with ECG. This is possible when the algorithm for IRE pulse delivery is based on early detection of the QRS complex, including the QR junction slope and R wave peak of the ECG signals, which is prior to the vulnerable period and is the safest time for pulse delivery.[73] This method of synchronizing IRE pulses with ECG has shown to be an effective and reliable tool for detecting QRS and preventing pulses from being delivered in case of abnormalities in heart rate.[58,73] Thomson et al[21] recently conducted a prospective, nonrandomized cohort study of IRE ablation of liver, kidney, and lung tumors in human patients. Four out of eight patients experienced transient ventricular arrhythmia when IRE was not synchronized with ECG. However, in procedures utilizing ECG synchronized IRE, there were only two cases of transient ventricular arrhythmia in a sample of 30 patients.[21] Thus, synchronization of pulse delivery with ECG presents a significant improvement from existing procedures of electroporation delivery with respect to the safety of the patient.[21,58]

Recently, we have conducted a preclinical study comparing the effectiveness and safety of the ECG synchronized and non-synchronized IRE ablation. The synchronized IRE ablation created a similar sized ablation zone compared with nonsynchronized IRE with a markedly improved safety profile (unpublished data). Even the ablation performed at the dome of the liver did not cause any dysrhythmias.

In summary, based on several clinical and preclinical studies, synchronized IRE is a safe and effective way to prevent any IRE-associated cardiac arrhythmia. However, this requires additional studies and therefore, each IRE ablation should be optimized based on each patient's cardiac history and risk stratification.

Pain control

Postoperative pain in IRE will be an important factor in overall patient satisfaction of the procedure. Narayanan et al,[75] whose past report treated over 100 liver cancer patients with IRE,[18] examined differences in self-reported pain between HCC patients treated with IRE versus those treated with RFA. The IRE treatment group consisted of 21 patients with a total of 29 intrahepatic lesions, whereas the RFA group included 22 patients with 27 foci of cancer. The level of pain experienced by each patient was assessed by self-evaluation and the cumulative application of patient-controlled analgesia (hydromorphone) in the 24 hours after surgery. Upon analysis, there were no significant differences in self-reported pain or the amount of self-injected analgesia between patients treated with IRE and those treated with RFA. These results suggest that patients will tolerate IRE tumor ablation at least as well as they tolerate modalities currently in use.[75] Kasivisvanathan et al[15] recently reaffirmed this conclusion by describing a case of successful use of IRE for the focal ablation of a tumor at the porta hepatis.

Research, clinical developments, and potential for oncological treatment

IRE is now being studied with great enthusiasm in several organ systems as a nonthermal, minimally invasive tissue ablation modality for ablating tumors and other undesired tissue with

promising preliminary data. After Davalos et al[2] demonstrated that IRE can be used to destroy substantial volumes of tissue without inducing thermally damaging effects, several researchers have confirmed these findings in small and large animal models in the liver,[2,3,6,7,76] breast,[77] prostate,[9] brain,[78–83] pancreas,[84,85] kidney,[86] lung,[87] and in implanted mouse sarcomas,[50,51] using a variety of pulse parameters.

Data from IRE performed in clinical *in vivo* human trials have been presented at several international conferences, published articles, and multiple case reports over the past few years, predominantly focusing on treatment of tumors within the liver, lung, prostate, pancreas, and kidney. These studies have evaluated the safety and feasibility of performing IRE for tissue ablation in human patients, showing a favorable safety profile and heralding the anticipation of further human studies on the way.

The following is a summary of oncologically driven preclinical and also clinical human studies using IRE for tumor ablation.

Liver and metastases to liver

Preclinical liver studies

Lee et al[88] demonstrated the efficacy of IRE in treating large hepatic tumors *in vivo* in a study consisting of 35 New Zealand white rabbits with VX2 tumors transplanted within their livers. Of these, 10 rabbits were subjected to single IRE application, 10 rabbits underwent multiple IRE applications, and 15 rabbits served as a control group. After all specimens in each treatment group had undergone ablation, the respective livers were evaluated with US, CT, and immunohistochemical analysis. Lee et al[88] found that multiple IRE applications were able to produce complete tumor ablation without any complications.[88]

Clinical liver studies

Kingham et al[16] conducted a 10-month retrospective study of 28 human patients treated with IRE for 65 malignant hepatic tumors. Patients underwent contrast-enhanced CT or MRI postoperatively, at 1–3 months, and at 6 months following the procedure. At 6 months, four tumors had experienced a recurrence. There was only one postoperative portal vein thrombosis despite the presence of 41 tumors within 1 cm of a major hepatic vein or portal pedicle. At 6 months, there was no mortality associated with the treatment. The authors concluded that IRE is a safe treatment option of perivascular malignant hepatic tumors that may not be treated with conventional modalities.[16]

Cannon et al[27] reported on IRE of hepatic tumors near vital structures, with 44 patients undergoing 48 total IRE procedures, including 20 colorectal metastases, 14 hepatocellular, and 10 other metastases. They reported initial success in all 46 (100%) of the treatments, with local recurrence free survival at 3 months, 6 months, and 12 months of 97.4%, 94.6%, and 59.5%, respectively. They noticed a trend toward higher recurrence rates with ablation of tumors over 4 cm (HR 3.236, 95% CI: 0.585–17.891; $P = 0.178$). Five patients had nine adverse events, of which three were thought to be possibly related to the IRE procedure, including neurogenic bladder, abdominal pain, and flank pain, although all complications resolved within 30 days.

Pancreas

Preclinical pancreatic studies

Prior to the advent of IRE, the range of options for the noninvasive treatment of locally advanced pancreatic cancer was essentially nonexistent due to the high risk of pancreatitis and damage to critical adjacent vessels.[85]

In a study by Charpentier et al,[84] four domestic female swine were subjected to IRE of a normal pancreas, using two monopolar probes spaced 9–15 mm apart. Ninety pulses of 1500 V/cm were delivered for each ablation, with no procedure related complications. Three animals in which probes had been spaced at intervals of 10 +/– 1 mm showed evidence of irreversible ablation by gross appearance and triphenyltetrazolium chloride (TTC) staining. The only animal in which probes had been spaced at intervals of 15 mm did not show evidence of irreversible ablation at 2 weeks. This was likely secondary to the relatively low voltage from wider probe spacing, which mainly results in reversible electroporation without cell death. These findings suggest that IRE appears to be a safe method for pancreas tissue ablation, with staining by TTC able to predict the extent of the IRE ablation zone within 2 hours of treatment.[84]

Bower et al[85] also explored the utility of IRE in the pancreas through a swine model. Four out of six total pigs successfully underwent IRE, whereas the procedure was aborted in two pigs because a stable current could not be maintained after multiple attempts. Pathological examination confirmed destruction of tissue in the ablation zone with sparing of surrounding vasculature. There were no complications other than transient increases in serum amylase and lipase postoperatively.

In a recent study, José et al[89] demonstrated the potential utility of IRE in treating pancreatic ductal adenocarcinoma (PDAC). Forty athymic mice were each implanted with 5×10^5 human pancreatic adenocarcinoma cell line BxPC-3 BxPC-3-Luc cells to generate orthotopic human pancreatic cancer xenografts. In the treatment group consisting of 24 mice, tumors were subject to IRE once they had grown to approximately 2–5 mm in diameter. The control group of 16 mice underwent a sham operation devoid of IRE. Twenty five percent of mice in the treatment group demonstrated complete tumor ablation. However, 19% of treatment mice indicated tumor regrowth at 30 days following surgery. Median survival time increased from 42 days in untreated mice to 88 days for mice who had undergone IRE. PDAC is devastating cancer with a 5-year survival rate that is less than 5%.[89] Though the results of this study are not perfect, they provide great hope for treatments of advanced human cancer cases that are currently inoperable.

Clinical pancreatic studies

In the first reported application of IRE to the treatment of human pancreatic cancer, a prospective multi-institutional pilot conducted by Martin et al[17] evaluated the utility of IRE in treating locally advanced pancreatic cancer. Of 27 patients who underwent treatment, 26 were treated invasively and one was treated percutaneously due to a history of multiple past surgeries in the same region. Nineteen patients were subject to *in situ* IRE, whereas the others' treatment regimen consisted of IRE in addition to either left-sided ($n = 4$) or pancreatic head ($n = 4$) resection. With the exception of a single mortality, there were no postoperative complications. All patients who completed 90-day follow-up experienced successful ablation of all tumors with no recurrences.[17] Though these results must be more conclusively established in larger scale trials, the success of this study indicate that IRE as one of only a few options available to patients with locally advanced pancreatic cancer. Following this study, Bagla and Papdouris[13] reported the application of percutaneous IRE to the treatment of a nonmetastatic, surgically unresectable pancreatic adenocarcinoma in a 78-year-old patient. As in the previous study, MRI confirmed a successful ablation with no recurrence and decreasing cancer antigen 19–9 level at 6-month-follow-ups.[13]

Narayanan et al[25] evaluated the use of IRE in the treatment of 14 patients for downstaging and control of unresectable locally advanced pancreatic cancer. Of these, three patients had metastatic cancer and were intolerant of chemotherapy, and 11 had disease localized to the pancreas. Previous to IRE, 11 patients had received radiation and three patients had received chemotherapy. Tumor sizes ranged from 2.5 cm to 7 cm with a median of 3.3 cm. Following IRE, all patients were found to have patent vasculature in the regions exposed to treatment. There were no deaths attributed directly to the IRE procedure, with spontaneous pneumothorax and pancreatitis representing the only complications encountered.[25]

Martin et al[26] recently reported results of a prospective, multi-institutional evaluation of 54 patients who underwent IRE for unresectable pancreatic cancer, including 35 patients with pancreatic head primary and 19 patients with body tumors. Of these, 19 patients underwent margin accentuation with IRE and 35 patients underwent *in situ* IRE. Forty nine (90%) patients had pre-IRE chemotherapy alone or chemoradiation therapy for a median duration 5 months, and 40 (73%) patients underwent post IRE chemotherapy or chemoradiation. The authors found that the 90-day mortality in the IRE patients was 1 (2%). Additionally, in a comparison of IRE patients to standard therapy, they report an improvement in local progression-free survival (14 months vs. 6 months, $P = 0.01$), distant progression-free survival (15 months vs. 9months, $P = 0.02$), and overall survival (20 months vs. 13months, $P = 0.03$), showing potential to achieve greater local palliation and potential in improved overall survival compared with standard chemoradiation–chemotherapy treatments.[26]

Lung

The proximity of the lung to the heart can pose a contraindication to ablative techniques such as RFA that result in central thermal sinks within the mediastinum. Because IRE is a nonthermal technique, its clinical implementation would present newfound hope for patients with thoracic tumors that are currently not candidates for surgical treatment.[90]

Preclinical lung studies

In the first study to evaluate the efficacy of IRE for the ablation of lung tissue, Dupuy et al[90] created 15 percutaneous fluoroscopy-guided IRE lesions in the lungs of nine anesthetized domestic swine. Radiographs and high-resolution CT were utilized to evaluate the animals prior to when each lung was harvested to facilitate histological examination. Microscopically, the parenchyma subject to IRE demonstrated well-demarcated alveolar damage with fibrosis and inflammatory infiltration. As expected, there was no damage to bronchioles and blood vessels within the ablative zone.[90] Within months of this initial study, Deodhar et al[87] conducted a similarly designed study, also involving swine, to reaffirm the successful use of IRE for the ablation of lung tissue.[87]

Clinical lung studies

Usman et al[22] reported the first two cases of the application of IRE to the treatment of lung neoplasms in humans. In the first case, a 33-year-old male presenting with recurrent pulmonary metastasis of synovial cell carcinoma was treated with IRE, in order to avoid collateral damage to adjacent pulmonary artery branches and lobar bronchi. A lesion measuring 2.3 cm × 2.4 cm × 1.7 cm was targeted and at the 2-month follow-up, the tumor had recurred, as evident by uniform enhancement on contrast CT, and increased metabolic activity on *positron emission tomography*. The second patient was a 70-year-old female who was found to have a slowly progressing tumor in the immediate suprahilar region, showing characteristics of both adenocarcinoma and squamous cell carcinoma. The tumor was located superior to the azygous vein and ventral to the trachea. Treatment of the 2.1 cm × 1.9 cm × 2.1 cm tumor with IRE proved unsuccessful at the 2-month follow-up. At 9 months, CT scan images suggested invasion of the tumor into the trachea. The patient passed away 1 year postoperation.[22] The small sample size of this report necessitates further investigations to determine whether IRE can treat lung cancer adequately, to prevent recurrences and raise the life expectancy in patients with no other surgical treatment options.[22]

Fanta et al[23] demonstrated the efficacy of IRE in the removal of central nonsmall cell lung tumors in two patients. In the first patient, a 3-cm epidermoid carcinoma completely obstructing the right main stem bronchus had resulted in atelectasis. Pneumonectomy was contraindicated due to poor lung function. The second patient suffered from a 2-cm carcinoid tumor extending from the right main stem bronchus to the carina, and preferred IRE due to the risk associated with resection. In both cases, IRE was performed through open thoracotomy with no complications. A CT scan demonstrated successful ablation in both patients, with no signs of recurrence present after 6 months.[23]

Renal

Preclinical renal studies

Tracy et al[91] subjected eight Yorkshire pigs to IRE of the kidney. Within 1 hour after ablation, renal cells in the zone of ablation were no longer viable, demonstrating diffuse desquamation of tubular cells, eosinophilia, and nuclear condensation. Comparison of successively harvested kidneys revealed that initially diffusely hemorrhagic legions progressed to smaller, acellular scars over the course of 14 days. Concurrently, periablative regions that had initially experienced patchy urothelial injury appeared to be undergoing a process of repair.[91]

Deodhar et al[86] conducted a similar study using a porcine model, to evaluate the effects of IRE ablation on normal renal tissue. CT-guided IRE was performed on 15 female swine to produce a total of 29 ablations. CT imaging immediately after the procedure revealed a hypodense nonenhancing lesion that persisted for up to 7 days after surgery. Connective tissue, including the pelvic extracellular matrix and blood vessels, as well as the renal pelvis and collecting system, remained intact in all cases.[86] Wendler et al[55] expounded on these studies by using MRI to evaluate the IRE ablation of renal tissue. Histological analysis of targeted tissue revealed IRE destruction of cortical glomeruli and tubules, but no damage to supporting structures including collecting ducts, renal calyxes, and the pelvis of medulla.[55]

Gastrointestinal (GI) tract

Preclinical GI Studies

The small intestine is particularly prone to collateral damage during invasive and noninvasive procedures targeting gastrointestinal cancer. Phillips et al[92] postulated that the tendency of IRE ablation to spare the extracellular matrix would allow quicker regeneration of mucosal cells. In turn, adverse side effects resulting from IRE ablation would be shorter-lived than traditional procedures. Thirteen Sprague–Dawley rats were anesthetized, and their intestines were exposed to 50–70 μs long 200 V pulses at a frequency of 4 Hz. Histological analysis revealed that all cells in the targeted area were completely ablated. Regeneration of the epithelial layer was observed within 3 days, with development of

distinct layers taking place by Day 7 postprocedure. These results suggest that IRE may eventually be utilized to ablate abdominal tumors in human patients with minimal collateral damage to adjacent tissues.[92]

Schoellnast et al[57] investigated the utility of IRE in ablating perirectal tissue and the associated pathological effects on the rectum wall. In six pigs, CT-guided IRE ablation was performed in the presence of water-filled endorectal coils that prevented displacement of the rectum wall. Five other pigs were subjected to CT-guided IRE without endorectal-assisted fixation of the rectum. Subsequent pathological evaluation revealed that inflammation and fibrosis was more superficial in pigs whose rectums had been mobile and allowed to contract than in pigs whose rectum was fixed. Specifically, almost all lesions found in pigs with mobile rectums were limited to the external layer of the muscularis propria, whereas all lesions in pigs with fixed rectums were transmural. The investigators concluded that by leaving the rectum mobile and able to contract, IRE ablation could be successfully performed on perirectal tissue with minimal damage to the rectum wall.[57]

Brain

Preclinical brain studies

Garcia et al[78] demonstrated the safety and efficacy of IRE in ablating canine brain tissue by performing IRE on the right temporal lobe of a beagle. The dog did not suffer any side-effects attributable to IRE, and histopathological examination revealed a sharp boundary between normal and ablated tissue.[78]

Ellis et al[81] built on the previous findings by successfully performing intracranial IRE ablation of normal canine brain tissue. The IRE electrodes were placed into a targeted area of the brain in three dogs and delivered a series of short and intense electric pulses with varying levels of voltage between dogs. An additional dog was treated at an extreme voltage to determine the upper safety limits of the procedure and one other dog was used as a sham control. US was used at the time of the procedure to visualize the lesions intraoperatively and MR imaging was used to estimate the volumes of ablated tissue postoperatively. The lesion volume was found to decrease with decreasing voltage of applied pulses and histological examination revealed cell death within the treated volume with a submillimeter transition zone between ablated and normal brain. There were no apparent complications in the three dogs subjected to therapeutic voltage ranges. The dog exposed to extreme voltages suffered nonselective necrosis in the entire treatment field, leading to arterial thrombosis and subsequent lacunar infarction.

In a later study, Garcia et al[80]_ENREF_58 were the first to utilize IRE for the treatment of a spontaneous, inoperable malignant intracranial glioma in a canine patient. In this case, IRE ablation successfully reduced tumor volume, producing a sufficient decrease in intracranial pressure and associated improvement of neurological function to warrant adjunctive fractionated radiotherapy. Serial MRI examinations post IRE confirmed full remission of the malignancy, and the eventual death of the dog was due to progressive radiation encephalopathy rather than complications from cancer. These findings suggest a role for IRE in the treatment of malignant brain tumors that might be inoperable according to the current standards of care_ENREF_58.[80]

Breast

Preclinical breast studies

In a recent study by Neal et al,[77] human breast cancer tumors were orthotopically implanted in the mammary fat pad of 11 female Nu/Nu mice that were divided into treatment and control groups. Seven tumor-bearing mice were treated with IRE and tumor regression was observed in five out of seven of the MDA-MB231 human mammary tumors in the course of 4 weeks after treatment, with continued growth in controls.[77] These findings suggest that IRE could be an advantageous alternative to surgical resection for breast conserving therapy.

Cervix

Preclinical cervix studies

Zhou et al[48] investigated the efficacy of IRE at inducing death of human cervical adenocarcinoma (HeLa) cells. The investigators found that either a low number of high-voltage pulses or a high number of low-voltage pulses were capable of causing HeLa cell death. A low number of pulses of high-voltage IRE resulted in necrosis of targeted human cervical adenocarcinoma cells. By contrast, a higher number of pulses of low-voltage IRE induced cell death through apoptosis, a preferred mechanism because of the lack of accompanying inflammation and potential for tissue regeneration posttherapy.[48]

Prostate

Preclinical prostate studies

Onik et al[9] studied IRE ablation in the normal prostate of six males dogs and demonstrated complete cell destruction within the IRE lesions, and rapid resolution of the lesions with marked shrinkage within 2 weeks. Structures such as urethra, vessels, nerves, and rectum were unaffected by the IRE application.[9]

Rubinsky et al[40] studied IRE ablation of prostate adenocarcinoma cells *in vitro* to determine the number, length, and field strength of IRE pulses required to produce complete human cancer cell ablation. They found that the upper and lower limit bounds of pulse length and number in a field range of 2000 V/cm to 2500 V/cm with a total of 90 pulses at 2500 V/cm for 100 ms separated by 100 ms could completely ablate prostate cancer cells without inducing thermal damage.[40] These findings suggest that IRE ablation of the prostate appears to be safe and have advantages in the clinical setting as compared to thermal ablation modalities, including preservation of important structures in this delicate part of the body.

Sarcoma

Preclinical sarcoma studies

Al-Sakere et al[51] studied the use of IRE for the minimally invasive treatment of aggressive cutaneous sarcoma tumors implanted in mice. Six mice, 6–8 weeks old, were inoculated subcutaneously in the left flank with cells from an LPB cell line, a methylcholanthrene-induced C57Bl/6 mouse sarcoma cell line, producing tumors of 4–5 mm in diameter in 9 days. They showed that successful outcome of the IRE procedure is related to the applied electric field strength, the total pulse duration, and temporal mode of pulse delivery. The best results were obtained using plate electrodes to deliver 80 pulses of 100 μs at 0.3 Hz with an electrical field magnitude of 2500 V/cm across the tumor. Tumor regression was confirmed by histological studies, with complete regression in 12 out of 13 treated tumors (92%) in the absence of tissue heating.[51]

Imaging and treatment evaluation

Lee et al[7] demonstrated that IRE can be performed as a real-time percutaneous image-guided intervention, with observation and measurements of the treated area acquired during real-time monitoring correlating well with pathological measurement of the lesion. This is in contrast to thermal ablation techniques such as RFA and cryoablation, which create hyperechoic microbubbles from the thermally injured tissue in US images, significantly hindering the possibility of real-time monitoring.[93,94]

Lee et al[6,7] showed that IRE creates a spherical hypoechoic area of ablation during and immediately after IRE in US images, with no lesion-obscuring hyperechoic gas, that lasts up to 24 hours after which the area of hypoechogenicity becomes hyperechogenic. They postulate that the initial hypoechogenicity is likely to be caused by increased intra/extracellular water content (edema and hyperemia) in the area of ablation as a result of the disruption of cellular homeostasis from the opening of transmembrane pores by the high voltage of electroporation.[6,7] Thus, ablation with IRE allows the operator to visualize the effects of IRE with real-time US, thus allowing for monitoring the effects of the electroporation pulses as they are applied, and ideally making it possible to adjust protocol parameters in real time to achieve desired results.

Following IRE, US representation of the ablation zone is dynamic and continues to evolve for hours following the conclusion of the procedure. Applebausm et al[95] performed *in vivo* ablation of 16 pig livers, and subsequently described the varying phases of the post IRE US ablation zone. Within minutes, an initially hypoechoic and well-demarcated zone gave way to a smaller and more isoechoic representation on US. After 25–90 minutes, hemorrhagic infiltration within the tissue led to the development of a peripheral hyperechoic rim around the isoechoic zone. Comparison to histological findings showed that the size of the external hyperechoic rim at 90–120 minutes is the best predictor of the ablation zone produced by IRE.[95] Similarly, Schmidt et al[96] affirmed that US after 24 hours is more precise than US after 6 hours at localizing cell death produced by IRE.[96]

Granot et al[97] recently described the use of three dimensional electrical impedance tomography (EIT) for real time monitoring of IRE, with histological analysis showing good correlation between the extent of tissue damage caused by IRE and EIT images. They report that EIT is the only imaging technique based on a measurement principle that is directly influenced by the electroporation phenomenon through measuring passive electrical properties of tissues. They argue that EIT may be the only feasible candidate for real time imaging of electroporation with feedback control purposes because other techniques, such as US, detect the indirect consequences of electroporation and thus may be too slow for a feedback scheme.[97]

Besides the use of these modalities for real time IRE ablation, treatment outcome and extent of IRE ablation can also be confirmed with CT and MRI, showing creation of discrete and measurable areas of ablation on CT and MR images in a 24-hour period.[6,78,97,98] Lee et al[6,88] have published multiple studies on radiologic–pathologic correlation of IRE-induced cell death. In a 2010 report,[6] US guided IRE of normal liver was performed on 16 Yorkshire pigs, 55 ablation zones were created and imaged with US, MRI, and CT and evaluated with immunohistochemical analysis. IRE ablation zones were well characterized with US, CT, and MR imaging, and real-time monitoring was feasible with US. IRE proved to be a fast, safe, and potent ablative method, causing complete tissue death with full preservation of periablative zone structures, including blood vessels, bile ducts, and neighboring nonablated tissues.[6]

Guo et al[99] recently demonstrated that like US, MR imaging might be useful for the quantitative measurement of the size of IRE ablation zones. Fifteen rats were injected with gadopentetate dimeglumine contrast agent, and subsequently subjected to IRE ablation of the liver. Two hours after the procedure, MRI measurements using conventional T1-weighted gradient echo sequence (GRE) and inversion recovery sequence (IR)-prepared GRE methods were performed. Upon harvesting of the livers, MRI results were correlated with histologically determined areas of cell death. The investigators found that cell death by IRE corresponds to hyperintense regions measured on T1-weighted GRE images. Once smaller hyperintense regions representing live penumbra tissue were subtracted from the T1-weighted GRE measurements, the remaining penumbra-nulled IR images proved to be very close approximations of pathology-confirmed ablation zones.[99]

Goldberg et al[100] previously demonstrated *in vitro* that the extent of decrease in the value of tissue galvanic apparent internal resistance (GAIR) during IRE treatment is indicative of the efficiency of IRE[100] In a more recent publication, Goldberg et al[101] examined the *in vivo* GAIR changes of rat liver hepatocytes exposed to IRE. The study found a 33% decrease in the value of GAIR immediately after the procedure, which increased to 40% at 3 hours following treatment. The authors suggest that once real-time monitoring of GAIR is feasible, the utilization of this method in clinical settings will allow intraprocedure evaluation of IRE efficacy.[101]

In conclusion, IRE is a novel minimally invasive tumor/tissue ablation technique with several recognized advantages: (1) preservation of surrounding vital structures such as vessels; (2) not affected by the heat-sink effect; (3) apoptotic, nonnecrotic, cell death with a quick tissue regeneration; (4) a shorter procedure time; and (5) able to perform under real-time monitoring. However, the history of IRE ablation research and development is still at its infancy and therefore, additional investigations are absolutely crucial to further understand and optimize this technique to improve the clinical outcomes and safety.

Conflicts of interest

All authors have no conflicts of interest to declare.

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