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ORIGINAL ARTICLE

# Irreversible electroporation (Nanoknife® treatment) in the field of hepatobiliary surgery: Current status and future perspectives

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## Summary

***Purpose:*** *We aimed to provide an overview of current understanding on the potential use of irreversible electroporation (IRE) in the field of hepatobiliary surgery with a focus on current results in hepatic and pancreatic cancers, its limitations, and its current directions.*

***Methods:*** *Through a review of the literature we have gathered the key articles and trials that are shaping our understanding of the current status of IRE and its prospective uses, and organized them in an easily understandable format showcasing the most up to date results.*

***Results:*** *IRE appears to be comparable in effectiveness and postoperative pain to the more established thermal ablation methods, while having the benefit of avoiding their detrimental thermal effects. In liver cancer, IRE was shown to be efficacious with low levels of local recurrences and only minimal complications. In pancreatic cancer it proved to have significant survival benefits but more significant (although rare) complications compared to the ones seen when IRE is used in liver cancer. Current evidence suggests a promising future for IRE, but clinical randomized control trials, and further developments of treatment protocols are required to come to more stable conclusions on the effectiveness and safety of IRE.*

***Conclusions:*** *IRE is proving to be an adequate method for the treatment of tumors of the pancreas and liver in cases where traditional methods are unavailable. It has been proven particularly efficacious in patients with masses in close proximity to vital structures such as vessels, as well as major biliary and hepatic structures where thermal methods of ablation would cause significant complications.*

***Key words:*** *ablation, hepatobiliary surgery, irreversible electroporation, liver cancer, pancreatic cancer, tumor*

## Introduction

The past 20 years have witnessed much research on the various ablative modalities for the treatment of focal liver and pancreatic tumors. This has led to tumor ablation being a recognized adjunct in the arsenal of hepatobiliary cancer treatment options for patients where surgical resection is not an option [1,2]. More specifically, ablation is now broadly indicated for patients with inoperable malignancies that do not show spread of the tumor to other parts of the body [3].

Through the use of local ablative modalities, various types of energy can be implemented to

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pass through a tumor in a controlled manner, so as to induce tissue destruction [4,5]. These ablative methods are grouped into thermal and non-thermal modalities which are separated based on the mechanism by which they induce cell death [4]. The most common thermal modality of ablation is Radiofrequency Ablation (RFA), while the most common non-thermal modality of ablation is IRE. Among others, RFA and IRE are reported as the most emerging local ablative methods used in hepatobiliary surgery [6].

Ablative techniques are used to cause a focal destruction of a tumor which in turn has the therapeutic potential to slow down disease progression and improve survival [4,7]. Within the field of hepatobiliary surgery, the aims of ablation include improving quality of life by cytoreduction leading to better symptom palliation, downstaging for subsequent resection, and prolonging survival [1,8,9].

Among hepatobiliary surgeons, IRE is of special interest as its non-thermal properties of ablation allow it to be implemented in anatomical regions which were previously considered inaccessible. More specifically, IRE is a modality of ablation which can be used in the treatment of tumors located near bile ducts and blood vessels which are ineligible for surgical resection or thermal ablation [1].

The IRE procedure itself is performed under general anesthesia, and can be done transcutaneously, laparoscopically, or by open procedure [1,3,10]. The full operation lasts between 2 to 4 hrs, with active ablation times lasting 2 to 3 min [3,10-12]. Under optimal circumstances, IRE allows patients to be discharged as early as the following day [3].

## Methods

The review of the literature focused on keyword searches of electronic databases, such as MEDLINE, Embase, Cochrane Library, and Google Scholar for articles dated past the year 2000. Our search terms included 'irreversible electroporation', 'pancreatic cancer', 'liver cancer', 'ablation', 'tumor', 'resection', 'indications', in various configurations. We selected relevant case series, retrospective studies, case-control studies, and narrative and systematic reviews. Through further review of the selected articles and hand-picked references, we formulated this narrative review.

## Results

*Irreversible electroporation*

IRE was first introduced as a method of tumor ablation by Roubinky's group in 2005 [10,13-15]. Until then, IRE was only known as an unintentional complication of reversible electroporation which was used in conjunction with cytotoxic drugs to treat cancer through electrochemotherapy [10,13]. This led to the first reported *in vivo* use of IRE on animals by Edd et al. in 2006 [14], and the first reported use of IRE on humans by Pech et al. in 2011 [11]. In 2007, Bertacchini et al. were the first to report an IRE system approved for clinical use [12].

Since then, multiple studies have demonstrated the efficacy of IRE in inducing cell death in a multitude of organs [10]. Animal studies have reported successful use of IRE for the ablation of cancers in the liver [13,14,16-18], pancreas [19,20], breast [21], prostate [22], kidney [23], lung [24], brain [25-30], and sarcomas [31,32]. In humans, IRE has been successfully used for the ablation of cancers in the liver [33,34], lung [35,36], pancreas [37-40], kidney [23,41,42], and prostate [43].

IRE works by manipulating the normal electric potential gradient which is present across all cellular membranes. Through creation of electric energy pulses, the transmembrane potential is affected leading to the disruption of the lipid bilayer. This then leads to the creation of permanent pores in the cell membrane which inhibit the cell's ability to maintain homeostasis and thus induces cell apoptosis [1,6,10,13-15,17,44-46].

The unique non-thermal properties of IRE lead to its ability to spare structures with high quantities of collagen and elastic fibers [10]. By keeping the collagen scaffold intact, vital structures such as the pancreatic ducts, bile ducts, portal triad, blood vessels, and nerves can be spared of destruction. Furthermore, these structures are then able to regenerate so as to bring back full function of the cells which were ablated [1,10,13-17,19,20,22,23,33,47-57]. An example of this property is nerve cells ablated through IRE showing a regenerative potential due to the retained intact architecture of the endoneurium and perineurium [1,10,52,55].

IRE is also not affected by heat sink, the cooling effect which is seen in thermal ablative methods mediated by adjacent blood flow in the area of ablation [48,51]. This is of crucial importance to hepatobiliary surgeons as IRE permits the ablation of tumors close to vascular structures [17,22,49,50], which is not an option with thermal ablative techniques due to heat sink.

Finally, IRE is a method which can be performed through minimally invasive procedures,

largely in part to the feasibility of periprocedural imaging [6,10]. Lee et al. [16,17] have demonstrated real-time ultrasound (US) image-guided percutaneous IRE in which a spherical hypoechoic area of ablation is created during and immediately after the procedure. The finding is reported to last 24 hrs before it turns from a hypoechoic area to a hyperechoic area [16,17]. In addition to real-time imaging, studies have also demonstrated the ability of computed tomography (CT) and magnetic resonance imaging (MRI) to demarcate measurable areas of ablation within the first 24 hrs after ablation [3,6,10,16,25,58-63].

Taking into account the non-thermal properties of IRE including the lack of heat sink, it's well demarcated histological borders, and its feasibility with the use of real-time US imaging, IRE enjoys the benefit of being able to be implemented in the ablation of much larger lesions in addition to causing much less complications compared to other ablative methods [10,13-15,17,44,50,64].

*IRE compared to thermal ablative methods*

As a non-thermal ablative method, IRE differs from other ablative methods in a number of ways. When compared to thermal ablative methods, such as RFA, IRE has a number of advantages that stem from the difference in the mechanism of induced cell death. Namely, IRE induces cell apoptosis compared to RFA which causes coagulative cell necrosis [10]. Apoptosis by IRE leads to cell removal by phagocytosis, meaning that the ability for innate cellular regeneration by surviving adjacent cells is retained and thus function may return in the ablated region [17,50]. Coagulative necrosis by thermal ablation does not posses this property as protein denaturation in conjunction with the subsequent scarring and fibrosis of the ablated region leads to tissue losing its potential for regeneration [17,50]. In addition to the retained regenerative potential, IRE also benefits from well demarcated borders of ablation which are not seen in thermal ablative methods where heat dissipates to adjacent tissue [17,50].

Some potential drawbacks of IRE which are not seen in other ablative methods stem from the powerful electric field which is required to be applied to the tumor [1]. In particular, these potential complications include cardiac arrhythmias and severe muscle contractions [1,66]. Measures do exist to reduce, or eliminate, the occurrence of these complications. Cardiac arrhythmias may be prevented through the careful use of electrocardiograph (ECG) synchronizers which allow for the administration of electrical field pulses in rhythm with the heart's refractory period [10-12,65-67]. The potential for severe muscle contractions may be reduced through the use of neuromuscular blocking agents, under general anesthesia, to prevent any muscle contractions [10,68]. This can be done during the interval of 90 to 100 IRE electrical pulses typically required for the full administration of ablation, which is synchronized to 90 to 100 heartbeats [10-12].

**Table 1.** Summary of major IRE trials for liver cancer

| *Authors* | *No. of patients* | *No. of lesions* | *Tumor type (No. of cases)* | *Primary efficacy %* | *Complications* | *Follow up time* |
|---|---|---|---|---|---|---|
| Cannon et al. [34] | 44 | 48 | HCC (14)<br>CRLM (20)<br>Other (10) | 97 | 5 | 12 months |
| Cheung et al. [72] | 11 | 18 | HCC (11) | 67 | 4 | 18 months |
| Kingham et al. [33] | 28 | 65 | HCC (11)<br>CRLM (21)<br>Other (5) | 96 | 4 | 6 months |
| Narayan et al. [70] | 21 | 29 | HCC (21) | - | 3 | - |
| Nissen et al. [75] | 1 | 1 | HCC (1) | 100 | 0 | - |
| Nissen et al. [74] | 1 | 1 | CRLM (1) | 100 | 0 | - |
| Scheffer et al. [76] | 10 | 10 | CRLM (10) | 90 | 0 | 4 weeks |
| Silk et al. [73] | 9 | 19 | CRLM (8)<br>Other (1) | - | 3 | 9 months |
| Sugimoto et al. [77] | 5 | 6 | HCC (6) | 83 | 0 | 9 months |
| Thomson et al. [67] | 13 | 45 | CRLM (6)<br>Other (7) | 67 | 2 | - |

CRLM:colorectal liver metastasis, HCC:hepatocellular carcinoma

Postoperative pain is a variable that has also been examined. Thus far, the research shows no significant difference in postoperative pain between patients that have undergone IRE and RFA ablation [69,70].

*IRE in the field of hepatobiliary surgery*

*Liver cancer*

IRE was found effective for ablating liver malignancies in preclinical studies. In a study of 35 New Zealand White Rabbits implanted with large VX2 liver tumor, those treated with multiple IRE cycles consistently showed complete cell death and complete tumor ablation [71].

In clinical studies IRE was also deemed to be efficacious (Table 1). In a retrospective study of 28 patients treated with IRE, only 4 out of the 65 tumors treated showed local recurrence at 6 months [33] and no mortality was associated with the procedure itself. Another study looking at 44 patients having undergone IRE for liver tumors and metastases near vital structures also showed a 97% initial procedure success rate without any mortality associated to the procedure itself [34]. This study also showed a 94% recurrence free survival at 6 months from the procedure, but that number dropped to 59.5% at the 12-month mark [34]. One other study of 11 patients treated for 18 lesions showed 6 local recurrences within an 18-month follow up [72].

Minimal complications were seen from IRE procedures. Amongst the studies looking at patients having undergone IRE, only few complications were reported, the majority of which were considered to be minor and unrelated to the IRE itself. The relevant complications related to IRE included pneumothorax, pleural effusion, hemothorax, transient arrhythmias, uncontrolled muscle contractions, transient increase in systolic blood pressure, pain, liver capsule puncture without subcapsular hemorrhage, neurogenic bladder, and different-sites pain [34,67,69,70,72-77].

*Pancreatic cancer*

Initially trialed in swine, IRE was found efficacious in producing irreversible cell death in healthy pancreatic tissue in two independent studies [19,20]. It was also observed that if the spacing between the probe was more than 15 mm with the lower voltage used, the electroporation was reversible [19]. A study in 40 mice implanted with human pancreatic ductal adenocarcinoma, 24 of which were treated with IRE, once the tumor grew to 2-5 mm in diameter a 25% complete ablation rate was noticed with an 18% recurrence rate, increasing the median survival from 42 days in the untreated group to 88 days in the IRE group [57].

Fewer clinical studies have been performed on the use of IRE for pancreatic cancer (Table 2). Martin et al. released the first trial of IRE on pancreatic cancer showing only one single 90-day mortality out of the 27 patients that underwent the intervention [37]. Also two studies by Narayanan et al. [39] with 14 patients and Martinet et al. [40] with 54 patients showed no mortality. A multicenter study by Philips et al. showed a demonstrable learning curve of at least 5 cases before becoming proficient with the use of IRE [78] suggesting that training has the capacity to decrease those rates.

Complications encountered in pancreatic IRE are incomplete ablation, duodenal leaks, pancreatitis, nausea/vomiting, infection, severe pain, DVT with PE, bile leak, biliary strictures, pancreatic abscess, and pancreaticoduodenal fistula [37,39,40,78-80]. A contraindication to the procedure would be prior presence of a metallic bile stent, as it could lead to perforation of the duodenum and colon, and potentially death by hemorrhage [80].

Mortality data from varying sources following IRE procedures, in addition to standard chemotherapy, have shown survival ranging from medians of 7.5 months to 24.9 months [37-40,78,79],

**Table 2.** Summary of major IRE trials for pancreatic cancer

| *Authors* | *No. of patients* | *Primary efficacy (%)* | *Complications (No. of patients)* | *Follow up time* |
|---|---|---|---|---|
| Bagla et al. [38] | 1 | 100 | 0 | 6 months |
| Martin et al. [37] | 27 | 96 | 4 | 90 days |
| Martin et al. [40] | 54 | 94 | 32 | 12 months |
| Martin et al. [79] | 200 | - | 74 | 20 months |
| Narayanan et al. [39] | 14 | 100 | 4 | 14 months |
| Paiella et al. [80] | 10 | 100 | 2 | 15 months |
| Philips et al. [78] | 59 | - | - | 18 months |

with the largest study of 200 patients showing a median survival of 24.9 months [79].

*Current status and future perspectives of IRE*

IRE is a relatively new ablative method that has not yet seen wide implementation and requires further research to examine its efficacy and safety. In the UK, IRE is only partly offered by the National Health Service (NHS) through few clinical trials, or can be performed privately at a price of roughly 15,000 pounds [3]. With regard to the current status of scientific knowledge on IRE, there is still insufficient evidence to safely come to conclusions about its long term benefits [6]. In addition, higher level research studies such as randomized controlled trials comparing IRE to other ablative methods are still not available in the literature [6]. A number of topics that are yet to be fully investigated and answered in IRE include its potential for adverse thermal effects, its necessity for an immune reaction following ablation, the potential use of concurrent electrochemotherapy, novel methods of monitoring ablation, and determining the ultimate treatment protocol.

Currently, IRE is described as a non-thermal ablative process, however tissue damage due to thermal energy has been reported in the literature [1,82]. More specifically, heat damage following IRE has been described immediately adjacent to the IRE electrodes [82], as well as around metallic stents which could be heated up due to the conductive nature of the metal [6,84]. As the possibility of thermal injury and occlusion of vital structures during IRE has not yet been ruled out, it has been recommended that electrodes should be placed at least 2 mm away from central bile ducts, pancreatic ducts, and intestinal tissue [1,73].

Another field of interest for its potentials in the use of IRE ablation is the molecular events that take place following the procedure. One such example is the involvement of the immune system after performing IRE [6]. While there is evidence to support enhanced immune antitumor stimulation after IRE [84,85], Al-Sakere et al. demonstrated a lack of local infiltration of tumor cells in the ablated tissue [31]. This is a point of interest as further evidence to support a lack of immune system involvement in the process of ablation could lead to the successful implementation of IRE in immunosuppressed patients [6]. An additional point of interest in the molecular level is the concurrent use of electrochemotherapy to kill any remnant tumor cells [1,86]. Successful use of concurrent electrochemotherapy has the potential for a reduced rate of recurrence, especially for larger tumors which currently show the greatest likelihood of local recurrence [33,34,67,72,73].

Further knowledge on the monitoring methods and their capabilities in IRE is also required. In addition to monitoring IRE ablation by US, CT, and MRI in the first 24 hrs, particular interest exists in the results of real-time monitoring modalities, and their subsequent correlations to long-term treatment outcomes [1,16,63,71,87]. Potential interest for IRE monitoring also exists in the measurement of changes in the electric conductivity of the ablated tissue. This is a technique reported as another potential mode of measuring the ablation effect [1,87-90].

One more frontier in our current knowledge of IRE with great potential to enhance our future implementation of this technique is the treatment protocol. The outcome of ablation by IRE depends on a number of IRE parameters (number, shape, and length of electrical pulses, interval between pulses, field amplitude, polarity) [10,91], and cell parameters (type, morphology, age, size) [10,15,92-97]. Determining the ideal IRE parameters for the treatment protocol by mathematical models has proven difficult, as tumor cell populations *in vivo* are never homogeneous and always in different stages of development [10,91]. This adds a considerable level of complexity in finding ideal IRE treatment protocols [10,91].

Last but not least, reports in the literature identify accurate electrode placement as the most challenging IRE parameter to optimize, even with an open surgical approach [10,98]. Updated electrodes, electrode stabilizers, and imaging guiding systems are currently being researched and their results are awaited [10].

## Conclusion

In conclusion, IRE appears to be a promising technique in the field of hepatobiliary surgery. It emerges as an adequate method for the treatment of tumors of the pancreas and liver in cases where traditional methods are unavailable or deemed to have a high risk for complications. IRE has been proven particularly efficacious in patients with masses in close proximity to vital structures such as vessels, as well as major biliary and hepatic structures. It is of major importance that IRE avoids thermal effects where traditional methods of ablation would cause significant complications related their thermal effects. As rigorous studies addressing much of the unknown variables left to

be polished, we strongly believe that IRE is set to become the next breakthrough in late-stage pancreatic and liver cancer treatment.

## Conflict of interests

The authors declare no confict of interests.

## References


1. Scheffer HJ, Nielsen K, de Jong MC et al. Irreversible electroporation for nonthermal tumor ablation in the clinical setting: a systematic review of safety and efficacy. J Vasc Interv Radiol 2014;25:997-1011; quiz 1011.
2. Ahmed M, Brace CL, Lee FT, Goldberg SN. Principles of and advances in percutaneous ablation. Radiology 2011;258:351-369.
3. Action PC. Irreversible Electroporation (NanoKnife®) · Pancreatic Cancer Action [Internet]. Pancreatic Cancer Action. [cited 2016 May 9]. Available from: https://pancreaticcanceraction.org/about-pancreatic-cancer/treatment/radiotherapy/irreversible-electroporation-nanoknife/
4. Petrou A, Moris D, Tabet PP, Richards BDW, Kourounis G. Ablation of the locally advanced pancreatic cancer: An introduction and brief summary of techniques. JBUON 2016;21:[In Press]
5. Rossi M, Orgera G, Hatzidakis A, Krokidis M. Minimally invasive ablation treatment for locally advanced pancreatic adenocarcinoma. Cardiovasc Intervent Radiol 2014;37:586-591.
6. Paiella S, Salvia R, Ramera M et al. Local Ablative Strategies for Ductal Pancreatic Cancer (Radiofrequency Ablation, Irreversible Electroporation): A Review. Gastroenterol Res Pract 2016;2016:4508376.
7. Rombouts SJE, Vogel JA, van Santvoort HC et al. Systematic review of innovative ablative therapies for the treatment of locally advanced pancreatic cancer. Br J Surg 2015;102:182-193.
8. Girelli R, Frigerio I, Salvia R, Barbi E, Tinazzi Martini P, Bassi C. Feasibility and safety of radiofrequency ablation for locally advanced pancreatic cancer. Br J Surg 2010;97:220-225.
9. Hadjicostas P, Malakounides N, Varianos C, Kitiris E, Lerni F, Symeonides P. Radiofrequency ablation in pancreatic cancer. HPB 2006;8:61-64.
10. Jourabchi N, Beroukhim K, Tafti BA, Kee ST, Lee EW. Irreversible electroporation (NanoKnife) in cancer treatment. Gastrointest Intervent 2014;3:8-18.
11. Pech M, Janitzky A, Wendler JJ et al. Irreversible electroporation of renal cell carcinoma: a first-in-man phase I clinical study. Cardiovasc Intervent Radiol 2011;34:132-138.
12. Bertacchini C, Margotti PM, Bergamini E, Lodi A, Ronchetti M, Cadossi R. Design of an irreversible electroporation system for clinical use. Technol Cancer Res Treat 2007;6:313-320.
13. Davalos RV, Mir ILM, Rubinsky B. Tissue ablation with irreversible electroporation. Ann Biomed Eng 2005;33:223-231.
14 Edd JF, Horowitz L, Davalos RV, Mir LM, Rubinsky B. In vivo results of a new focal tissue ablation technique: irreversible electroporation. IEEE Trans Biomed Eng 2006;53:1409-1415.
15. Miller L, Leor J, Rubinsky B. Cancer cells ablation with irreversible electroporation. Technol Cancer Res Treat 2005;4:699-705.
16. Lee EW, Chen C, Prieto VE, Dry SM, Loh CT, Kee ST. Advanced hepatic ablation technique for creating complete cell death: irreversible electroporation. Radiology 2010;255:426-433.
17. Lee EW, Loh CT, Kee ST. Imaging guided percutaneous irreversible electroporation: ultrasound and immunohistological correlation. Technol Cancer Res Treat 2007;6:287-294.
18. Guo Y, Zhang Y, Klein R et al. Irreversible electroporation therapy in the liver: longitudinal efficacy studies in a rat model of hepatocellular carcinoma. Cancer Res 2010;70:1555-1563.
19. Charpentier KP, Wolf F, Noble L, Winn B, Resnick M, Dupuy DE. Irreversible electroporation of the pancreas in swine: a pilot study. HPB (Oxford). 2010;12:348-351.
20. Bower M, Sherwood L, Li Y, Martin R. Irreversible electroporation of the pancreas: definitive local therapy without systemic effects. J Surg Oncol 2011;104:22-28.
21. Neal RE, Singh R, Hatcher HC, Kock ND, Torti SV, Davalos RV. Treatment of breast cancer through the application of irreversible electroporation using a novel minimally invasive single needle electrode. Breast Cancer Res Treat 2010;123:295-301.
22. Onik G, Mikus P, Rubinsky B. Irreversible electroporation: implications for prostate ablation. Technol Cancer Res Treat 2007;6:295-300.
23. Deodhar A, Monette S, Single GW et al. Renal Tissue Ablation With Irreversible Electroporation: Preliminary Results in a Porcine Model. Urology 2011;77:754-760.
24. Deodhar A, Monette S, Single GW et al. Percutaneous irreversible electroporation lung ablation: preliminary results in a porcine model. Cardiovasc Intervent Radiol 2011;34:1278-1287.
25. Garcia PA, Rossmeisl JH, Robertson J, Ellis TL, Davalos RV. Pilot study of irreversible electroporation for intracranial surgery. In: 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 2009, pp 6513-6516.
26. Garcia PA, Neal RE, Rossmeisl JH, Davalos RV. Non-thermal irreversible electroporation for deep

intracranial disorders. In: 2010 Annual International Conference of the IEEE Engineering in Medicine and Biology, 2010, pp 2743-2746.

27. Garcia PA, Pancotto T, Rossmeisl JH et al. Non-thermal irreversible electroporation (N-TIRE) and adjuvant fractionated radiotherapeutic multimodal therapy for intracranial malignant glioma in a canine patient. Technol Cancer Res Treat 2011;10:73-83.
28. Ellis TL, Garcia PA, Rossmeisl JH, Henao-Guerrero N, Robertson J, Davalos RV. Nonthermal irreversible electroporation for intracranial surgical applications. J Neurosurg 2010;114:681-688.
29. Garcia PA, Rossmeisl JH, Davalos RV. Electrical conductivity changes during irreversible electroporation treatment of brain cancer. In: 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 2011, pp 739-742.
30. Hjouj M, Last D, Guez D et al. MRI Study on Reversible and Irreversible Electroporation Induced Blood Brain Barrier Disruption. PLoS One 2012;7(8):e42817.
31. Al-Sakere B, Bernat C, Andre F et al. A study of the immunological response to tumor ablation with irreversible electroporation. Technol Cancer Res Treat 2007;6:301-306.
32. Al-Sakere B, André F, Bernat C et al. Tumor Ablation with Irreversible Electroporation. PLoS One [Internet]. 2007 Nov 7 [cited 2016 May 18];2(11). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2065844/
33. Kingham TP, Karkar AM, D'Angelica MI et al. Ablation of Perivascular Hepatic Malignant Tumors with Irreversible Electroporation. J Am Coll Surg 2012;215:379-387.
34. Cannon R, Ellis S, Hayes D, Narayanan G, Martin RCG. Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. J Surg Oncol 2013;107:544-549.
35. Usman M, Moore W, Talati R, Watkins K, Bilfinger TV. Irreversible electroporation of lung neoplasm: a case series. Med Sci Monit 2012;18:CS43-47.
36. Fanta J, Horák P, Marvan J et al. The NanoKnife and two successful cases of intracavitary irreversible electroporation of main bronchus tumors. Rozhl Chir 2012;91:625-630.
37. Martin RCG, McFarland K, Ellis S, Velanovich V. Irreversible Electroporation Therapy in the Management of Locally Advanced Pancreatic Adenocarcinoma. J Am Coll Surg 2012;215:361-369.
38. Bagla S, Papadouris D. Percutaneous Irreversible Electroporation of Surgically Unresectable Pancreatic Cancer: A Case Report. J Vasc Intervent Radiol 2012;23:142-145.
39. Narayanan G, Hosein PJ, Arora G et al. Percutaneous Irreversible Electroporation for Downstaging and Control of Unresectable Pancreatic Adenocarcinoma. J Vasc Intervent Radiol 2012;23:1613-1621.
40. Martin RCG, McFarland K, Ellis S, Velanovich V. Irreversible electroporation in locally advanced pancreatic cancer: potential improved overall survival. Ann Surg Oncol 2013;20 (Suppl 3):S443-449.
41. Tracy CR, Kabbani W, Cadeddu JA. Irreversible electroporation (IRE): a novel method for renal tissue ablation. BJU International 2011;107:1982-1987.
42. Wendler JJ, Porsch M, Hühne S et al. Short- and mid-term effects of irreversible electroporation on normal renal tissue: an animal model. Cardiovasc Intervent Radiol 2013;36:512-520.
43. Scheltema MJV, van den Bos W, de Bruin DM et al. Focal vs extended ablation in localized prostate cancer with irreversible electroporation; a multi-center randomized controlled trial. BMC Cancer 2016;16:299.
44. Rubinsky B. Irreversible electroporation in medicine. Technol Cancer Res Treat 2007;6:255-260.
45. Esser AT, Smith KC, Gowrishankar TR, Weaver JC. Towards solid tumor treatment by irreversible electroporation: intrinsic redistribution of fields and currents in tissue. Technol Cancer Res Treat 2007;6:261-274.
46. Esser AT, Smith KC, Gowrishankar TR, Weaver JC. Towards Solid Tumor Treatment by Nanosecond Pulsed Electric Fields. Technol Cancer Res Treat 2009;8:289-306.
47. Adam R, Hagopian EJ, Linhares M et al. A comparison of percutaneous cryosurgery and percutaneous radiofrequency for unresectable hepatic malignancies. Arch Surg 2002;137:1332-1339.
48. Goldberg SN, Hahn PF, Tanabe KK et al. Percutaneous Radiofrequency Tissue Ablation: Does Perfusion-mediated Tissue Cooling Limit Coagulation Necrosis? J Vasc Intervent Radiol 1998;9:101-111.
49. Maor E, Ivorra A, Leor J, Rubinsky B. The Effect of Irreversible Electroporation on Blood Vessels. Technol Cancer Res Treat 2007;6:307-312.
50. Rubinsky B, Onik G, Mikus P. Irreversible Electroporation: A New Ablation Modality; Clinical Implications. Technol Cancer Res Treat 2007;6:37-48.
51. Charpentier KP, Wolf F, Noble L, Winn B, Resnick M, Dupuy DE. Irreversible electroporation of the liver and liver hilum in swine. HPB 2011;13:168-173.
52. Schoellnast H, Monette S, Ezell PC et al. Acute and Subacute Effects of Irreversible Electroporation on Nerves: Experimental Study in a Pig Model. Radiology 2011;260:421-427.
53. Li W, Fan Q, Ji Z, Qiu X, Li Z. The effects of irreversible electroporation (IRE) on nerves. PLoS One 2011;6(4):e18831.
54. Phillips MA, Narayan R, Padath T, Rubinsky B. Irreversible electroporation on the small intestine. Br J Cancer 2012;106:490-495.
55. Schoellnast H, Monette S, Ezell PC et al. Irreversible electroporation adjacent to the rectum: evaluation of pathological effects in a pig model. Cardiovasc Intervent Radiol 2013;36:213-220.
56. Wendler JJ, Pech M, Porsch M et al. Urinary Tract Effects After Multifocal Nonthermal Irreversible Electroporation of the Kidney: Acute and Chronic Monitoring by Magnetic Resonance Imaging, Intravenous Urography and Urinary Cytology. Cardiovasc Intervent Radiol 2011;35:921-926.
57. José A, Sobrevals L, Ivorra A, Fillat C. Irreversible electroporation shows efficacy against pancreatic car-

cinoma without systemic toxicity in mouse models. Cancer Lett 2012;317:16-23.

58. Granot Y, Ivorra A, Maor E, Rubinsky B. In vivo imaging of irreversible electroporation by means of electrical impedance tomography. Phys Med Biol 2009;54:4927.
59. Zhang Y, Guo Y, Ragin AB et al. MR Imaging to Assess Immediate Response to Irreversible Electroporation for Targeted Ablation of Liver Tissues: Preclinical Feasibility Studies in a Rodent Model. Radiology 2010;256:424-432.
60. Mahmood F, Hansen RH, Agerholm-Larsen B, Jensen KS, Iversen HK, Gehl J. Diffusion-weighted MRI for verification of electroporation-based treatments. J Membr Biol 2011;240:131-138.
61. Guo Y, Zhang Y, Nijm GM et al. Irreversible electroporation in the liver: contrast-enhanced inversion-recovery MR imaging approaches to differentiate reversibly electroporated penumbra from irreversibly electroporated ablation zones. Radiology 2011;258:461-468.
62. Kranjc M, Markelc B, Bajd F et al. In Situ Monitoring of Electric Field Distribution in Mouse Tumor during Electroporation. Radiology 2014;274:115-123.
63. Lee YJ, Lu DSK, Osuagwu F, Lassman C. Irreversible electroporation in porcine liver: acute computed tomography appearance of ablation zone with histopathologic correlation. J Comput Assist Tomogr 2013;37:154-158.
64. Lavee J, Onik G, Mikus P, Rubinsky B. A novel nonthermal energy source for surgical epicardial atrial ablation: irreversible electroporation. Heart Surg Forum 2007;10:E162-167.
65. Deodhar A, Dickfeld T, Single GW, et al. Irreversible Electroporation Near the Heart: Ventricular Arrhythmias Can Be Prevented With ECG Synchronization. Am J Roentgenol 2011;196:W330-335.
66. Ball C, Thomson KR, Kavnoudias H. Irreversible electroporation: a new challenge in "out of operating theater" anesthesia. Anesth Analg 2010;110:1305-1309.
67. Thomson KR, Cheung W, Ellis SJ et al. Investigation of the safety of irreversible electroporation in humans. J Vasc Interv Radiol 2011;22:611-621.
68. Golberg A, Rubinsky B. Towards electroporation based treatment planning considering electric field induced muscle contractions. Technol Cancer Res Treat 2012;11:189-201.
69. Kasivisvanathan V, Thapar A, Oskrochi Y, Picard J, Leen ELS. Irreversible electroporation for focal ablation at the porta hepatis. Cardiovasc Intervent Radiol 2012;35:1531-1534.
70. Narayanan G, Froud T, Lo K, Barbery KJ, Perez-Rojas E, Yrizarry J. Pain analysis in patients with hepatocellular carcinoma: irreversible electroporation versus radiofrequency ablation-initial observations. Cardiovasc Intervent Radiol 2013;36:176-182.
71. Lee EW, Wong D, Tafti BA et al. Irreversible Electroporation in Eradication of Rabbit VX2 Liver Tumor. J Vasc Intervent Radiol 2012;23:833-840.
72. Cheung W, Kavnoudias H, Roberts S, Szkandera B, Kemp W, Thomson KR. Irreversible electroporation for unresectable hepatocellular carcinoma: initial experience and review of safety and outcomes. Technol Cancer Res Treat 2013;12:233-241.
73. Silk MT, Wimmer T, Lee KS et al. Percutaneous Ablation of Peribiliary Tumors with Irreversible Electroporation. J Vasc Intervent Radiol 2014;25:112-118.
74. Niessen C, Jung E, Stroszczynski C, Wiggermann P. Ablation of liver metastasis with irreversible electroporation (IRE) in segment II of the liver adjacent to the non-peritoneal area. RÖFO 2012;184:937-938 (in German).
75. Niessen C, Jung EM, Wohlgemuth WA et al. Irreversible Electroporation of a Hepatocellular Carcinoma Lesion Adjacent to a Transjugular Intrahepatic Portosystemic Shunt Stent Graft. Korean J Radiol 2013;14:797-800.
76. Scheffer HJ, Nielsen K, van Tilborg AA et al. Ablation of colorectal liver metastases by irreversible electroporation: results of the COLDFIRE-I ablate-and-resect study. Eur Radiol 2014;24:2467-2475.
77. Sugimoto K, Moriyasu F, Kobayashi Y et al. Irreversible electroporation for nonthermal tumor ablation in patients with hepatocellular carcinoma: initial clinical experience in Japan. Jpn J Radiol 2015;33:424-432.
78. Philips P, Hays D, Martin RCG. Irreversible electroporation ablation (IRE) of unresectable soft tissue tumors: learning curve evaluation in the first 150 patients treated. PLoS One. 2013;8(11):e76260.
79. Martin RCG, Kwon D, Chalikonda S et al. Treatment of 200 locally advanced (stage III) pancreatic adenocarcinoma patients with irreversible electroporation: safety and efficacy. Ann Surg 2015;262:486-494; discussion 492-494.
80. Paiella S, Butturini G, Frigerio I et al. Safety and feasibility of Irreversible Electroporation (IRE) in patients with locally advanced pancreatic cancer: results of a prospective study. Dig Surg 2015;32:90-97.
81. Mansson 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 Rep 2014;3(11):2047981614556409.
82. Faroja M, Ahmed M, Appelbaum L et al. Irreversible electroporation ablation: is all the damage nonthermal? Radiology 2013;266:462-470.
83. Dunki-Jacobs EM, Philips P, Martin RCG. Evaluation of thermal injury to liver, pancreas and kidney during irreversible electroporation in an in vivo experimental model. Br J Surg 2014;101:1113-1121.
84. Li X, Xu K, Li W et al. Immunologic response to tumor ablation with irreversible electroporation. PLoS One 2012;7(11):e48749.
85. Neal RE, Rossmeisl JH, Robertson JL et al. Improved local and systemic anti-tumor efficacy for irreversible electroporation in immunocompetent versus immunodeficient mice. PLoS One 2013;8(5):e64559.
86. Charpentier KP. Irreversible electroporation for the ablation of liver tumors: are we there yet? Arch Surg 2012;147:1053-1061.
87. Appelbaum L, Ben-David E, Sosna J, Nissenbaum Y, Goldberg SN. US findings after irreversible electro-

poration ablation: radiologic-pathologic correlation. Radiology 2012;262:117-125.

88. Ivorra A, Al-Sakere B, Rubinsky B, Mir LM. In vivo electrical conductivity measurements during and after tumor electroporation: conductivity changes reflect the treatment outcome. Phys Med Biol 2009;54:5949-5463.
89. Pavlin M, Kanduser M, Rebersek M et al. Effect of cell electroporation on the conductivity of a cell suspension. Biophys J 2005;88:4378-4390.
90. Glahder J, Norrild B, Persson MB, Persson BRR. Transfection of HeLa-cells with pEGFP plasmid by impedance power-assisted electroporation. Biotechnol Bioeng 2005;92:267-276.
91. Golberg A, Rubinsky B. A statistical model for multidimensional irreversible electroporation cell death in tissue. BioMedical Engineering OnLine 2010;9:13.
92. Chen C, Smye SW, Robinson MP, Evans JA. Membrane electroporation theories: a review. Med Bio Eng Comput 2006;44:5-14.
93. Ho SY, Mittal GS. Electroporation of Cell Membranes: A Review. Crit Rev Biotechnol 1996;16:349-362.
94. Chang DC, Reese TS. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. Biophysical J 1990;58:1-12.
95. Kinosita K, Tsong TY. Formation and resealing of pores of controlled sizes in human erythrocyte membrane. Nature 1977;268:438-441.
96. Grahl T, Märkl H. Killing of microorganisms by pulsed electric fields. Appl Microbiol Biotechnol 1996;45:148-157.
97. Kinosita K, Tsong TY. Voltage-induced pore formation and hemolysis of human erythrocytes. Biochim Biophys Acta 1977;471:227-242.
98. Ben-David E, Appelbaum L, Sosna J, Nissenbaum I, Goldberg SN. Characterization of Irreversible Electroporation Ablation in In Vivo Porcine Liver. Am J Roentgenol 2012;198:W62-68.