

Percutaneous Ablation of Hepatic Tumors Using Irreversible Electroporation: A Prospective Safety and Midterm Efficacy Study in 34 Patients

Christoph Niessen, Dr Med, Lukas P. Beyer, Dr Med, Benedikt Pregler, MD, Marco Dollinger, Dr Med, Benedikt Trabold, PD Dr, Hans J. Schlitt, Prof Dr, Ernst M. Jung, Prof Dr, Christian Stroszczynski, Prof Dr, and Philipp Wiggermann, PD Dr

ABSTRACT

Purpose: To evaluate the safety and efficacy of percutaneous irreversible electroporation (IRE) of primary and secondary liver cancer unsuitable for resection or thermal ablation.

Materials and Methods: In this prospective, single-center study, 65 malignant liver tumors (hepatocellular carcinoma, $n = 33$; cholangiocellular carcinoma, $n = 5$; colorectal cancer metastasis, $n = 22$; neuroendocrine cancer metastasis, $n = 3$; testicular cancer metastasis, $n = 2$) in 34 patients (27 men, 7 women; mean age, $59.4 \text{ y} \pm 11.2$) were treated. Local recurrence-free survival (LRFS) according to the Kaplan-Meier method was evaluated after a median follow-up of 13.9 months.

Results: Median tumor diameter was $2.4 \text{ cm} \pm 1.4$ (range, 0.2–7.1 cm). Of 65 tumors, 12 (18.5%) required retreatment because of incomplete ablation ($n = 3$) or early local recurrence ($n = 9$). LRFS at 3, 6, and 12 months was 87.4%, 79.8%, and 74.8%. The median time to progressive disease according to modified Response Evaluation Criteria In Solid Tumors was 15.6 months. Overall complication rate was 27.5% with six major complications and eight minor complications. Major complications included diffuse intraperitoneal bleeding ($n = 1$), partial thrombosis of the portal vein ($n = 1$), and liver abscesses ($n = 4$). Minor complications were liver hematomas ($n = 6$) and clinically inapparent pneumothoraces ($n = 2$).

Conclusions: IRE showed promising results regarding therapeutic efficacy for the percutaneous treatment of liver tumors; however, significant concerns remain regarding its safety.

ABBREVIATIONS

CTCAE = Common Terminology Criteria for Adverse Events, IRE = irreversible electroporation, LRFS = local recurrence-free survival, mRECIST = modified Response Evaluation Criteria In Solid Tumors

From the Departments of Radiology (C.N., L.P.B., B.P., M.D., E.M.J., C.S., P.W.), Anesthesiology (B.T.), and Surgery (H.J.S.), University Hospital Regensburg, Franz-Josef-Strauss-Allee 11, Regensburg D-93053, Germany. Received July 17, 2015; final revision received December 8, 2015; accepted December 20, 2015. Address correspondence to C.N.; E-mail: christoph.niessen@ukr.de

None of the authors have identified a conflict of interest.

© SIR, 2016

J Vasc Interv Radiol 2016; 27:480–486

<http://dx.doi.org/10.1016/j.jvir.2015.12.025>

Because of its theoretical safety advantage over thermal ablative techniques, irreversible electroporation (IRE) has gained popularity for percutaneous tumor ablation, and it is currently used to treat tumors in locations where thermal ablation is contraindicated. However, its possible benefits should not be overestimated. Despite the assumption of the nonthermal nature of IRE, it has been shown that, if parameters are not chosen correctly, IRE may produce sufficient heat to induce coagulation necrosis under some conditions of high intensity (1–3). Moreover, the current IRE technology appears to be substantially affected by tissue properties and structure,

which might also influence the size and shape of the ablation area and hinder complete tumor destruction. In addition, the size of the ablation zone depends on many technical parameters, such as electrode spacing, relative position of electrodes, length of the active tip, pulse number and duration, and applied voltage. For this reason, the precise placement of at least two (usually four to six) electrodes in parallel is technically more challenging compared with conventional ablation techniques and may raise further challenges for effective tumor ablation (4).

Nonetheless, preclinical studies using animal models have shown the efficacy of IRE for ablation of hepatic tissue (5) and hepatocellular carcinoma (6). However, clinical data regarding safety and efficacy of IRE in the treatment of liver tumors in humans are limited (7–9). Scheffer et al (10) were the first to prove the histopathologic efficacy of IRE in humans with colorectal liver metastases in an ablate and resect trial. They found IRE to cause avitality of tumor cells within the ablation zones 1 hour after treatment. The aim of the present study was to contribute to current knowledge by prospective evaluation of the safety and of midterm efficacy of percutaneous IRE in patients with primary and secondary liver cancer in a clinical setting.

MATERIALS AND METHODS

Approval of the institutional review board was obtained for this prospective single-center study. From December 2011 to June 2013, 65 target tumors in 34 patients with primary or secondary liver cancer were percutaneously treated in 51 procedures (Table 1). Seven women (20.6%) and 27 men (79.4%) were included. Mean age of the patients was 59.4 years \pm 11.2 (range, 22–81 y). Patients were selected for IRE if surgical resection or thermal ablation was precluded, and each patient's case was discussed in a multidisciplinary tumor conference to ensure that all treating physicians from the disciplines

Table 1. Demographic Data

Variable	Value	Range
Total no. patients	34	
Median follow-up (mo)	13.9	1.8–19.5
Treated tumors	65	
Tumors treated per patient	1.91	1–4
Mean age (y)	59.4	22–81
Sex		
Men	27 (79.4%)	
Women	7 (20.6%)	
ECOG/Karnofsky performance status		
ECOG 1 (100%–70%)	23 (67.7%)	90%–40%
ECOG 2 (60%–50%)	8 (23.5%)	
ECOG 3 (40%–30%)	3 (8.8%)	

ECOG = Eastern Cooperative Oncology Group.

of medical oncology, radiation oncology, gastroenterology, interventional radiology, nuclear medicine, and surgery agreed with the proposed treatment plan. Table 2 shows inclusion and exclusion criteria for this prospective study.

The most frequent diagnoses were hepatocellular carcinoma (n = 15 patients, n = 33 tumors) and colorectal liver metastases (n = 12 patients, n = 22 tumors). Other tumor types included cholangiocellular carcinoma (n = 4 patients, n = 5 tumors), metastasis of testicular cancer (n = 1 patient, n = 2 tumors), and metastatic neuroendocrine tumors (n = 2 patients, n = 3 tumors). Before IRE treatment, complete staging consisting of contrast-enhanced computed tomography (CT) scan of the chest, abdomen, and pelvis and dedicated magnetic resonance (MR) imaging of the liver with gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Primovist; Bayer Pharma AG, Berlin, Germany) was performed during the patients' admission examinations (Fig 1).

Tumor Characteristics

The median largest diameter of the target lesions before ablation was 2.4 cm \pm 1.4 (minimum 0.2 cm, maximum 7.1 cm) with a mean volume of 10.2 cm³ \pm 17.0 (minimum 0.13 cm³, maximum 124.1 cm³). Of the 65 target lesions, 29 tumors were located in segments II, III, IVa, and IVb; 25 tumors were located in segments V and VI; and the remaining 11 tumors were located in segments VII and VIII (Table 3).

Demographic Data

Prior therapies of patients included surgical treatment (20 patients; 58.8%); systemic therapy (15 patients; 44.1%); and liver-directed therapies, such as radiofrequency ablation (seven patients; 20.6%), hepatic arterial therapy (four patients; 11.8%), and radiation therapy (three patients; 8.8%). Most patients with hepatocellular carcinoma had preserved liver function: seven patients with Child-Pugh class A (46.7%), six patients with Child-Pugh class B (40.0%), and two patients with Child-Pugh class C (13.3%).

Electroporation Protocol

The IRE procedures were performed with the Nano-Knife device (AngioDynamics, Latham, New York) and were carried out in accordance with the manufacturer's guidelines. Patients received general anesthesia, mechanical ventilation, and neuromuscular blocking. Treatment planning was based on the measurements of CT imaging performed before the intervention. Depending on tumor size and shape, the desired zone of tissue ablation to ensure a 1-cm safety margin around the entire tumor was entered into the generator. The number of required IRE electrodes (range, two to six) and their relative position to each other were planned on the IRE device.

Table 2. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
<ol style="list-style-type: none"> 1. Diagnosis of primary or secondary liver cancer based on positive biopsy result or noninvasive criteria (1 tumor < 5 cm, 3 tumors < 3 cm)* 2. Noncandidacy for conventional thermal ablation because of tumor location: <ol style="list-style-type: none"> a) Tumors located in proximity to bile duct, major hepatic artery or vein, or major portal vein branch (distance < 0.5 cm) b) Subcapsular or centrally located tumors or tumors adjacent to other organs (ie, gallbladder, stomach, colon) 3. Age 18–85 y 4. Male or female 5. Written informed consent 	<ol style="list-style-type: none"> 1. Resectable disease, which is defined as the possibility of completely removing all tumors and retaining a sufficient liver remnant to maintain liver function 2. Severe coagulation disorders (platelet count < 50,000/mm³; PTT > 50 s, INR < 1.5) 3. Presence of vascular invasion, multifocal hepatic disease, or extrahepatic spread on imaging 4. Previous treatment of target nodule 5. Patients who received systemic chemotherapy within 30 d of treatment with IRE 6. Severe heart failure, recent myocardial infarction, coronary artery disease, arrhythmia in progress, active implantable devices (eg, pacemaker) 7. Pregnancy or women of childbearing age not using contraception

INR = international normalized ratio; IRE = irreversible electroporation; PTT = partial thromboplastin time.

*One patient was treated with percutaneous IRE and included in this study because of the lack of therapy alternatives and strong patient wishes.

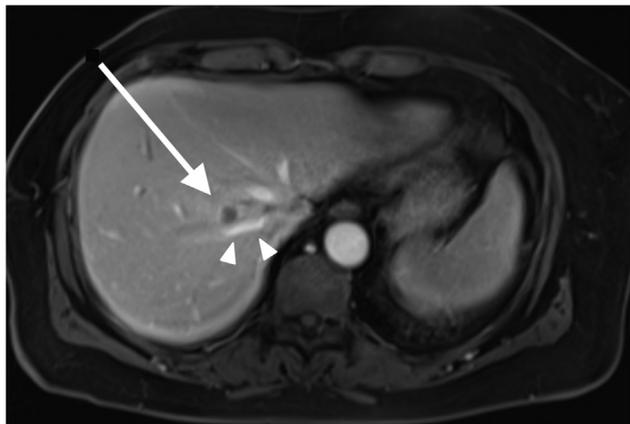


Figure 1. A 56-year-old man with colorectal carcinoma. Contrast-enhanced MR imaging during portal-venous phase after injection of 12 mL gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid performed 24 hours before ablation using IRE showing the tumor in segment VIII (arrow) adjacent to the right hepatic vein (arrowheads).

IRE electrodes were percutaneously placed into the target area under CT fluoroscopy (CARE Vision, SOMATOM 16; Siemens Healthcare GmbH, Erlangen, Germany) and high-definition ultrasound guidance using a multifrequency probe (1–5 MHz; Logiq E9; GE Healthcare, Marlborough, Massachusetts). The probes are placed optimally when they are oriented in parallel direction with a distance of 1.0–2.0 cm with the tips of the monopolar electrodes encompassing the tumor (Fig 2). The treatment parameter for voltage depended on the distance between the probes within the targeted tissue. After verification of

the correct electrode position, a 270-V test pulse was applied to confirm adequate tissue conductivity. Application of therapeutic electrical impulses to the target lesion was automatically synchronized with the patient's cardiac cycle (11). According to the manufacturer's instructions, standard treatment parameters were 70 pulses of 1,500 V/cm and 90 μ s.

According to the proposed standardization of terms and reporting criteria of the Working Group on Image-Guided Tumor Ablation (12), technical success was defined as the successful delivery of all planned pulses to the target volume as calculated by the IRE generator and complete tumor coverage, which was assessed by MR imaging or CT performed 6 weeks after ablation. After delivery of these impulses, the generator provides an ablation protocol with measurements of amperage between every electrode pair. The target current for tumor ablation is between 20 A and 50 A.

Follow-up, Therapy-Associated Side Effects, and Tumor Response

Before discharge, patients underwent imaging with both MR imaging and contrast-enhanced CT scans within 24 hours after the ablation. The purpose of this first follow-up imaging was primarily to evaluate for complications and not to evaluate treatment efficacy. Complications were evaluated in accordance with the criteria established by the Society of Interventional Radiology (SIR) (13) and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse

Events (CTCAE) (14). For evaluation of technical success, follow-up imaging was initiated 6 weeks after intervention; evaluation of tumor response was done at 3 months after the intervention and then at 3-monthly intervals (Fig 3). Tumor response was evaluated as overall response and as tumor response of target lesions according to the modified Response Evaluation Criteria In Solid Tumors (mRECIST). Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced MR imaging was the standard imaging modality for follow-up. The contrast-enhancement pattern was the determining factor of success or failure of tumor ablation.

Statistical Analysis

Local recurrence-free survival (LRFS) was determined according to the Kaplan-Meier method. LRFS was determined from the date of ablation to the date of radiographic local recurrence of the target lesion. In cases of incomplete ablation that required salvage treatment as documented during the 6-week follow-up, the period from the date of the salvage ablation was used. All statistical analyses were performed using Microsoft

Excel 2011 and IBM SPSS Statistics Version 20.0 for Mac (IBM Corporation, Armonk, New York).

RESULTS

Technical Aspects

Complete ablation as documented immediately after the intervention and during the 6-week follow-up was achieved in 62 of treated 65 tumors (95.4%). Two to six electrodes (mean of 3.1 electrodes per patient) were needed to ensure complete ablation. The mean duration of the procedures was 163.5 minutes \pm 59.5 (range, 62–400 min), measured from the time of first electrode placement to the time of final control CT scan.

Therapy-Associated Side Effects and Complications

In 51 IRE procedures, 14 complications (27.5%) occurred (Table 3). In accordance to the classification established by SIR (13), six complications were rated as major (11.8%), and eight complications were rated as minor (15.7%). Major complications included partial thrombosis of the portal vein in a patient with liver cirrhosis with the left branch of the portal vein abutting the tumor in segment IVa (distance < 0.5 cm), which required moderate anticoagulation of the patient (CTCAE 3). Another major complication was diffuse intraperitoneal bleeding that ceased spontaneously but required blood transfusion and admission to the intensive care unit (CTCAE 3). Four liver abscesses occurring after the intervention were documented; two required CT-guided percutaneous drainage in addition to systemic antibiotics (CTCAE 3), which were routinely administered only in patients with bilioenteric anastomosis (14). All abscesses occurred within the ablation zone and were detected on CT scan performed after the intervention. In three of four cases of liver abscess, the patients had a bilioenteric anastomosis.

Table 3. Tumor Characteristics

Variable	Value	Range
Largest median diameter (cm)	2.4	0.2–7.1
Mean volume (cm ³)	10.2	0.13–124.1
Location of tumor		
Segments II/III/IVa/IVb	29	
Segments V/VI	25	
Segments VII/VIII	11	
Tumor type		
Metastatic colorectal cancer	22 (33.8%)	
Hepatocellular carcinoma	33 (50.8%)	
Cholangiocellular carcinoma	5 (7.7%)	
Other	5 (7.7%)	

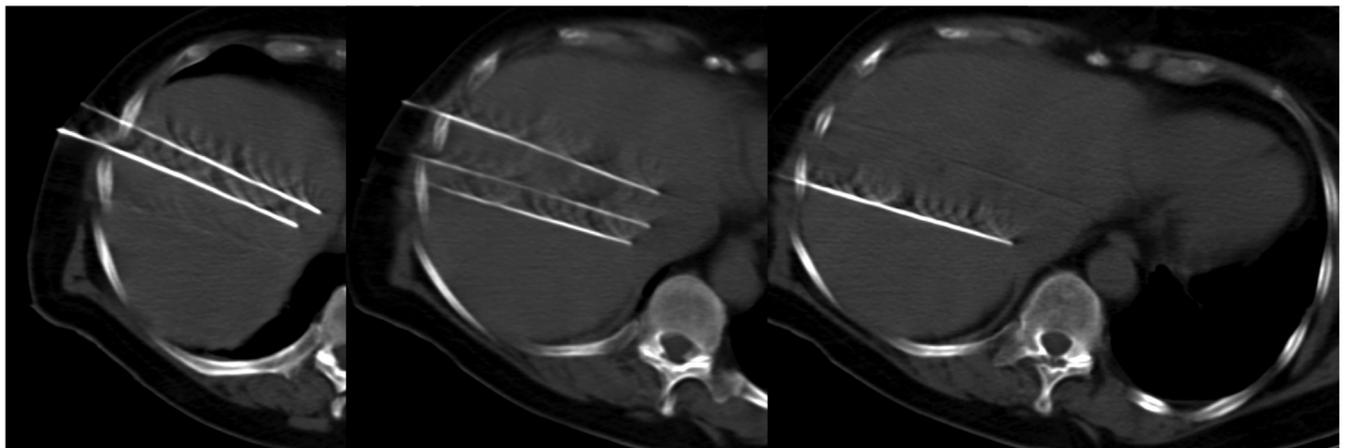


Figure 2. Unenhanced CT scan of the same patient (Fig 1) during the intervention. Control scan for planning IRE showing five IRE electrodes in parallel direction encompassing the tumor in segment VIII. To calculate technical IRE parameters, probe distances have to be entered into the generator's planning tool.

Minor complications were hematomas (n = 6; CTCAE 1) and small, clinically inapparent pneumothoraces (n = 2; CTCAE 1) that did not require any further therapy (14). There was no correlation between the occurrence of complications and the histologic tumor type or the location of the tumor or preceding chemotherapy (Table 4).

Tumor Response and Survival

A second treatment was required in 12 tumors because of incomplete ablation (n = 3) as documented during the 6-week follow-up or early local recurrence (n = 9) as documented during the 3-month and 6-month follow-up. All tumors with incomplete ablation were colorectal liver metastases with short-axis diameters of 3.0 cm, 2.6 cm, and 2.3 cm. Of the nine tumors with early local recurrence after 3 months (n = 6) and 6 months (n = 3), seven tumors were colorectal liver metastases, one was cholangiocellular carcinoma, and one was hepatocellular carcinoma. The follow-up period was 1.8–19.8 months, with median follow-up of 13.9 months. LRFS at 3 months, 6 months, and 12 months was 87.4%,

79.8%, and 74.8% (Fig 4). The mean time to local recurrence was 15.5 months. One of the 34 study patients died 9.8 months after the first intervention. The median time to progressive disease according to mRECIST criteria was 15.6 months. The percentage of patients with complete remission (complete response according to mRECIST) after 3 months, 6 months, and 12 months was 86.6%, 74.2%, and 61.9% (Fig 5).

DISCUSSION

IRE is currently undergoing clinical investigation as an alternative ablation technique that relies on electric instead of thermal energy for achievement of cell death. All types of normal and pathologic tissue as well as blood vessels are destroyed with thermal ablation, whereas IRE appears to be more selective; the technique has proven to be particularly effective in tissues with cell membrane structures of a high density, which is a common aspect of most malignant tissues, and less effective in connective tissue, as there are cells with a high concentration of collagenous and elastic fibers (15). IRE also seems to be unaffected by blood flow and, conversely, not to compromise the functionality of blood vessels in the treated area (9,16) with complete ablation up to the margin of blood vessels (16,17). The potential of axonal regeneration after treatment of the sciatic nerve using IRE was shown in a porcine model (18).

Davalos, Mir, and Rubinsky (19,20) showed through mathematical analysis that—in theory—IRE can be used for ablating substantial volumes of tissue. Their findings were subsequently confirmed in preclinical studies on hepatocarcinoma cells (HepG2) where IRE produced complete cancer cell destruction (21). Lee et al (5) treated normal livers of 16 Yorkshire pigs and showed that IRE produced irreversible tissue damage. However, data regarding the clinical outcome of IRE are limited. The first publication regarding therapeutic efficacy of hepatic IRE was published by Kingham et al (9). In their retrospective review of 65 patients treated mainly with an open surgical approach, LRFS was 94.4% after a median follow-up of 6 months. Similar results were found in the prospective analysis of a multicenter registry by Cannon et al (8), who reported overall LRFS after hepatic IRE at 3 months, 6 months, and 12 months of 97.4%, 94.6%, and 59.5%. There is a remarkable discrepancy between those results and the present study results regarding LRFS (79.8% and 74.8% after 6 and 12 months). The discrepancy can be at least partly attributed to the procedural approach, as one third of patients in the registry were treated via an open approach, and to the low number of patients in these three studies (Kingham et al, 28 patients and 65 tumors; Cannon et al, 44 patients and 46 tumors; present study, 34 patients and 65 tumors). Furthermore, the preceding studies did not define clearly how patients were chosen

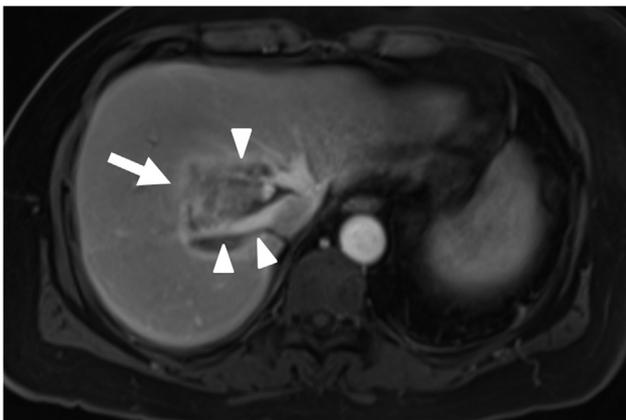


Figure 3. Same patient (Figs 1, 2) 6 months after the intervention. Contrast-enhanced MR imaging during portal-venous phase after injection of 12 mL gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid. Hypointense demarcation of ablation area (arrow) in segment VIII with hemorrhage following the intervention and perifocal hyperemia. Hepatic veins within the ablation area were not compromised by IRE treatment (arrowheads).

Table 4. Complications

Complications	Value
All complications	14 (27.5%)
Major complications	6 (11.8%)
Intraperitoneal bleeding	1 (2.0%)
Partial thrombosis of portal vein	1 (2.0%)
Abscess	4 (7.8%)
Minor complications	8 (15.7%)
Hematoma	6 (11.8%)
Pneumothorax	2 (3.9%)

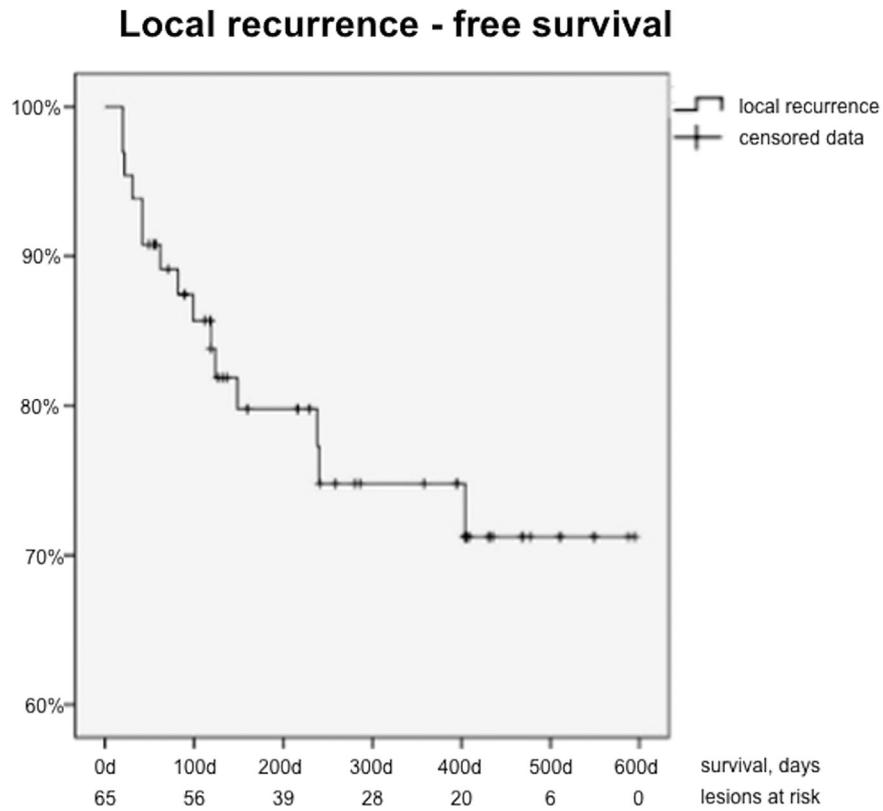


Figure 4. Kaplan-Meier analysis for local recurrence-free survival. Censored data stem from patients without any local recurrence at the end of the study.

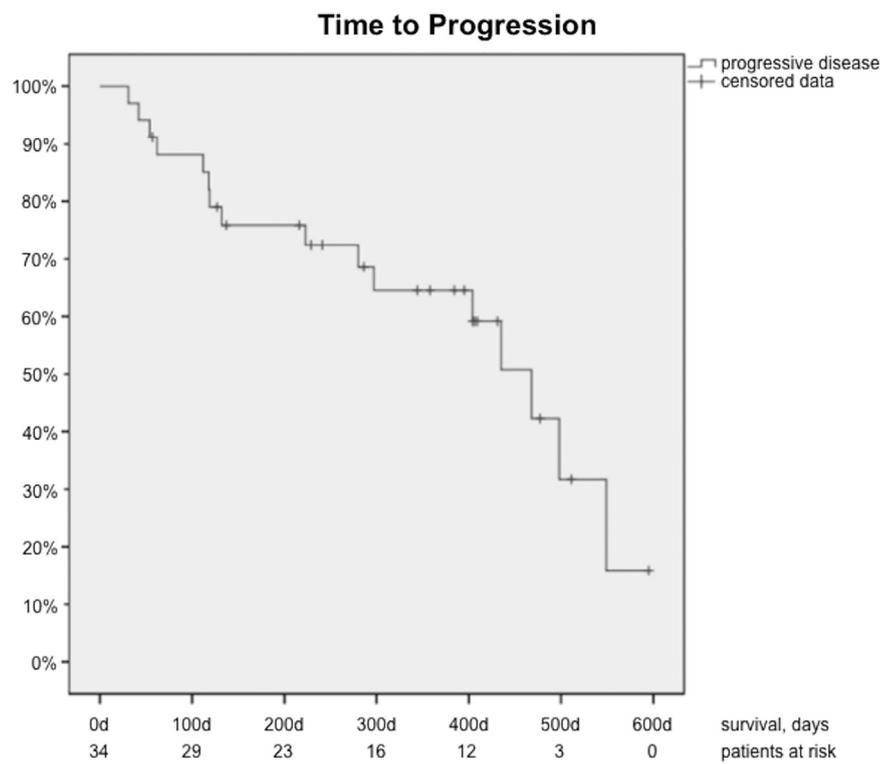


Figure 5. Kaplan-Meier analysis for time to progressive disease according to mRECIST.

for IRE procedures. In the present study, only patients who were poor candidates for thermal techniques were treated, and thus the IRE procedures of the tumors might have been more challenging, which also might have contributed to the lower LRFS rate in the current study.

Large tumor volumes are often associated with incomplete ablation and higher recurrence rates (22) after thermal ablation. This limitation of locally ablative techniques is—with restrictions—also applicable for IRE. In their clinical study, Cannon et al (8) showed an increased risk of recurrence for tumors with a diameter > 3 cm and recommended IRE as salvage therapy for tumors < 3–4 cm in diameter situated in locations that make them poor candidates for thermal ablation. Although it was not the primary goal of their prospective nonrandomized cohort study, Thomson et al (7) found no significant treatment effect for liver tumors > 5 cm in diameter. The aim of their study was to assess the safety profile of IRE. The authors did not observe any mortality in 38 patients with 69 tumors in the liver, kidneys, or lungs within 30 days after treatment. After the application of electrocardiogram-synchronized IRE impulses, two patients experienced arrhythmia, and two patients experienced temporary neurapraxia as a result of patient positioning with arm extension during a prolonged period of anesthesia (7). In their study, no treatment-associated deaths were recorded within 30 days after the intervention. In the present study, the complication rate of 27% appears to be relatively high, but 16% were minor complications that did not require any further therapy. However, similar results regarding therapy-associated side effects were found by Philips, Hays, and Martin (23), who assessed 150 consecutive patients treated by IRE via either a percutaneous or a surgical approach and found a complication rate of 29.3%.

The need of general anesthesia associated with a long duration of treatment procedures is seen as a major disadvantage of IRE when compared with radiofrequency ablation or microwave ablation. The mean duration of treatment in this study was almost 3 hours (163.5 minutes ± 59.5), which appears quite long but is similar to the study by Philips et al (23), who reported a median procedure time of 152.5 minutes.

This study had several limitations. A mixed patient cohort (ie, patients with different tumor types and different stages of disease) was included in this study. Other limitations were the relatively short follow-up period with a median follow-up of 1 year and prospective, single-arm study design.

In conclusion, the results of this study, in which only tumors ineligible for thermal ablation or resection were treated, show that IRE is a feasible and effective instrument in the growing toolbox of interventional radiologists. However, significant concerns remain regarding its safety. Because of its innovative ablation mechanism, IRE has the potential to widen the field of percutaneously

treatable liver tumors. IRE is not meant to replace thermal techniques, but rather to extend the spectrum of percutaneously treatable tumors.

REFERENCES

1. Faroja M, Ahmed M, Appelbaum L, et al. Irreversible electroporation ablation: is all the damage nonthermal? *Radiology* 2013; 266:462–470.
2. Dunki-Jacobs EM, Philips P, Martin RC 2nd. 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.
3. Wagstaff PG, de Bruin DM, van den Bos W, et al. Irreversible electroporation of the porcine kidney: temperature development and distribution. *Urol Oncol* 2015; 33:e1–e7.
4. Ben-David E, Ahmed M, Faroja M, et al. Irreversible electroporation: treatment effect is susceptible to local environment and tissue properties. *Radiology* 2013; 269:738–747.
5. 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.
6. 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.
7. 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.
8. Cannon R, Ellis S, Hayes D, Narayanan G, Martin RC 2nd. Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures. *J Surg Oncol* 2013; 107:544–549.
9. 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.
10. 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.
11. Deodhar A, Dickfeld T, Single GW, et al. Irreversible electroporation near the heart: ventricular arrhythmias can be prevented with ECG synchronization. *AJR Am J Roentgenol* 2011; 196:W330–W335.
12. Ahmed M, Solbiati L, Brace CL, et al. Image-guided tumor ablation: standardization of terminology and reporting criteria—a 10-year update. *J Vasc Interv Radiol* 2014; 25:1691–1705.e4.
13. Sacks D, McClenny TE, Cardella JF, Lewis CA. Society of Interventional Radiology clinical practice guidelines. *J Vasc Interv Radiol* 2003; 14: S199–S202.
14. U.S. Department of Health and Human Services; National Institutes of Health; National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). 2010. Available at: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Accessed October 15, 2015.
15. Maor E, Ivorra A, Rubinsky B. Non thermal irreversible electroporation: novel technology for vascular smooth muscle cells ablation. *PLoS One* 2009; 4:e4757.
16. Maor E, Ivorra A, Leor J, Rubinsky B. The effect of irreversible electroporation on blood vessels. *Technol Cancer Res Treat* 2007; 6:307–312.
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. 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.
19. Davalos RV, Mir LL, Rubinsky B. Tissue ablation with irreversible electroporation. *Ann Biomed Eng* 2005; 33:223–231.
20. 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.
21. Miller L, Leor J, Rubinsky B. Cancer cells ablation with irreversible electroporation. *Technol Cancer Res Treat* 2005; 4:699–705.
22. Mulier S, Ni Y, Jamart J, Ruers T, Marchal G, Michel L. Local recurrence after hepatic radiofrequency coagulation: multivariate meta-analysis and review of contributing factors. *Ann Surg* 2005; 242:158–171.
23. Philips P, Hays D, Martin RC. Irreversible electroporation ablation (IRE) of unresectable soft tissue tumors: learning curve evaluation in the first 150 patients treated. *PLoS One* 2013; 8:e76260.