A prospective Phase 2a pilot study investigating focal percutaneous irreversible electroporation (IRE) ablation by NanoKnife in patients with localised renal cell carcinoma (RCC) with delayed interval tumour resection (IRENE trial)

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A B S T R A C T

Introduction: Focal ablation therapy is playing an increasing role in oncology and may reduce the toxicity of current surgical treatments while achieving adequate oncological benefit. Irreversible electroporation (IRE) has been proposed to be tissue-selective with potential advantages compared with current thermal-ablation technologies or radiotherapy. The aim of this pilot trial is to determine the effectiveness and feasibility of focal percutaneous IRE in patients with localised renal cell cancer as a uro-oncological tumour model.

Methods: Prospective, monocentric Phase 2a pilot study following current recommendations, including those of the International Working Group on Image-Guided Tumor Ablation. Twenty patients with kidney tumour (T1aN0M0) will be recruited. This sample permits an appropriate evaluation of the feasibility and effectiveness of image-guided percutaneous IRE ablation of locally confined kidney tumours as well as functional outcomes. Percutaneous biopsy for histopathology will be performed before IRE, with magnetic-resonance imaging one day before and 2, 7, 27 and 112 days after IRE; at 28 days after IRE the tumour region will be completely resected and analysed by ultra-thin-layer histology.

Discussion: The IRENE study will investigate over a short-term observation period (by magnetic-resonance imaging, post-resection histology and assessment of technical feasibility) whether focal IRE, as a new ablation procedure for soft tissue, is feasible as a percutaneous, tissue-sparing method for complete ablation and cure of localised kidney tumours. Results from the kidney-tumour model can provide guidance for designing an effectiveness and feasibility trial to assess this new ablative technology, particularly in uro-oncology.

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Abbreviations: BfArM, German Federal Institute for Drugs and Medical Devices; CE, Community of Europe; CRF, case report form; CT, computer tomography; DIMDI, German Institute of Medical Documentation and Information; DRKS, German Clinical Trials Registry; EAU, European Association of Urology; ECG, electrocardiogram; ECOG, Eastern Co-operative Oncology Group; EORTC, European Organisation for Research and Treatment of Cancer; EUDAMED, European Databank on Medical Devices; FACT, Functional Assessment for Cancer Therapy; FKSI, FACT Kidney Symptoms Index; GCP, Good Clinical Practice; ICTRP, International Clinical Trials Registry Platform; IIT, investigator-initiated trial; IRE, irreversible electroporation; IRENE, IrReversible Electroporation of kidney tumours before partial NephrEctomy; KKS, German network of co-ordination centres for clinical studies; MPG, German Medical Devices Act; MRI, magnetic-resonance Imaging; NIH, U.S. National Institute of Health; OVGU, Otto von Guericke University of Magdeburg Germany; QLQ, Quality-of-Life Questionnaire; RCC, renal cell carcinoma; SAE, severe adverse event; TSC, Trial Steering Committee; US, ultrasound; UTN, Universal Trial Number; WHO, World Health Organisation.

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1. Introduction

Focal ablation therapy is playing an increasingly important role in oncology and may reduce the toxicity of current surgical treatments while achieving complete tumour destruction with a satisfactory oncological outcome. Newer ablation modalities, such as irreversible electroporation (IRE), have been introduced and their respective clinical niches are being defined [1]. IRE causes cell death through the repeated application of short-duration high-voltage electrical pulses that create irreversible damage to cell membranes [1,2]. While there may be some hyperthermic ablative changes with high-power applications, the mechanism of cell death with IRE is thought to be predominantly non-thermal [2,3,18,42].

In urology, the surgery as the standard treatment is moving toward minimally invasive procedures [4]. In localised renal cell carcinoma, partial nephrectomy should be performed whenever technically feasible, so as to avoid chronic renal failure [4]. For this reason, cryoablation and radiofrequency ablation (RFA) have been put forward as alternatives by the EAU for small renal masses (<4 cm) in selected patients [4]. IRE has been proposed to be tissue-selective and possibly to convey advantages compared with currently used thermal ablative technologies or radiotherapy [5]. Therefore, IRE represents an interesting option for nephron-sparing treatment of renal tumours, even for those with unfavourable anatomic location (e.g., centrally located and close to the renal pelvis and/or the large hilar vessels).

Conclusive reports on the use of IRE in kidney malignancies, especially renal cell carcinoma, are not yet available. Previous investigations on renal IRE were exclusively experimental or safety-oriented [6–21]. A complete histological analysis is still required to assess the completeness of tumour ablation and the nephron-sparing potential of IRE in RCC. Studies published so far – mainly case reports or series reports of patients undergoing palliative treatment – do not permit systematic analysis. However, one of the criticisms of published ablation studies in general is the sparse tissue outcome data, because most studies are based only on radiographic or post-biopsy assessments [1]. With this study (the “IRENE study”) we present the first detailed histopathological data on the short-term effect of IRE-treated renal tumours in humans after secondary surgical tumour resection. To the best of our knowledge this is the first study determining the safety and effectiveness of IRE of localised renal cell carcinomas with curative treatment intention.

2. IRENE study protocol

2.1. Study title for registration

“Prospective, monocentric clinical Phase 2a study of the effectiveness of the percutaneous irreversible electroporation (IRE) as primary ablation therapy of locally confined kidney tumours (renal cell carcinomas).” Acronym and short title “IRENE – IReversible Electroporation of kidney tumours before partial NephreCtoMy” [22–24].

2.2. Study registration

The trial has been registered as follows:

- ClinicalTrials.gov of the U.S. National Institutes of Health: NCT01967407 [22].
- International Clinical Trials Registry Platform (ICTRP) of the World Health Organisation (WHO): DRKS00004266 [23].
- German Clinical Trials Registry (DRKS): DRKS00004266 [24].
- German Federal Institute for Drugs and Medical Devices (BfArM) and German Institute of Medical Documentation and Information (DIMDI) with the European Databank on Medical Devices (EUDAMED) number: CIV-12-04-006021.

- Study protocol number for the ethics committee responsible: MD-UBO-001.

2.3. Study approval

The study was registered at the German Institute of Medical Documentation and Information (DIMDI). Approval for this study was granted by the BfArM and the Ethics Committee of the Medical Faculty of the Otto von Guericke University of Magdeburg (OVGU) [no. 73/12]. The NanoKnife System has been CE-certified for the ablation of soft tissue (all parenchymatous organs and tumours of the soft tissues) under the number CE 0086 (formerly CE 0050 Generator and CE 0051 electrodes) [25]. The system is classified as a Class II device according to the Risk Classification for Medicinal Products of the FDA and as belonging to Class IIb according to the European regulation 93/42/EGW.

2.4. Study management

IRENE is an interventional, investigator-initiated trial (IIT), compliant with Good Clinical Practice (GCP) and the German Medical Devices Act (MPG) guidelines as well as with those of the International Working Group on Image-Guided Tumor Ablation [1]. Investigators from the University Hospital designed the protocol under due consideration of feedback from the Ethics Committee of the OVGU and the Clinical Study Centre of the Medical Faculty of the OVGU. The study is sponsored by the Medical Faculty of the OVGU, and will be conducted at the University Hospital in Magdeburg. The Trial Steering Committee (TSC) is composed of an independent chairperson, the principal investigator, the study co-ordinator, a patient representative, a leading study nurse, the leading co-investigators of the both clinical departments involved, all other co-investigators and the study statistician. The independent data-monitoring committee includes independent urologists, radiologists and pathologists in the field of tumour therapy and focal therapy (all of whom are independent of the study and are reviewers of the German academies of focal therapy and microtherapy).

2.5. Trial design

This is a prospective, monocentric, non-randomised, uncontrolled, non-blinded, single-arm Phase 2a interventional pilot study to assess the clinical effectiveness of a novel 2007 CE-certified procedure and medicinal product (NanoKnife Electroporation Ablation System, AngioDynamics Inc., Latham, NY 12110, USA). In spite of the strict selection criteria and anticipated slow recruitment, the duration of the study has at present been set to two years. The follow-up period is 4 months after IRE.

2.6. Study rationale

Animal experiments, clinical case reports and safety application studies all indicate that the use of IRE to treat kidney tumours is safe and without relevant side effects. Furthermore, experiments have shown that IRE efficiently ablates target tissue. Thorough histological investigations following complete tumour resection by IRE in localised kidney tumours are not described in the literature. Therefore, post-procedural imaging findings provide only a rough guide to the success of ablation therapy, because microscopic foci of residual disease cannot be expected to be identified by standard imaging [1,26]. The gold standard for treatment of localised RCC is surgical resection, increasingly as nephron-sparing surgery. Therefore, in this study the tumour ablation region will be removed surgically four weeks after IRE. Imaging, in conformity with the relevant guidelines (EAU [4]), will be conducted by repeated MRI and results will be compared with histological findings.
The study is intended to reveal the current value of percutaneous IRE, guided by CT and/or sonography with MRI-fusion.

2.7. Aim of the study

The objective of the study is to evaluate percutaneous IRE as a novel ablation procedure performed with the only electroproporation system at present available for this the NanoKnife Electroporation Ablation System (AngioDynamics Inc., Latham, NY 12110, USA) in the treatment of localised kidney tumours with subsequent operative tumour resection (partial kidney resection) after four weeks. The success of IRE will be assessed by MRI and histological testing (short-term and medium-term effects). The hypothesis that kidney tumours of ≤4 cm can be completely ablated will be tested. The sample size will be 20 (see Section 2.17).

2.7.1. Primary objective

— To assess the “probable oncotherapeutic effectiveness”, measured by the proportion of residual viable tumour in the kidney, as revealed by histopathology and imaging 28 days after IRE.

2.7.2. Secondary objective

— To assess the technical feasibility of percutaneous IRE performed with the NanoKnife (see above) and guided by CT and/or sonography with MRI fusion, under clinical conditions, on human kidney tumours.

— To assess, 28 days after IRE, by imaging and histology, peritumoural tissue damage during nephron-sparing focal tumour ablation.

— To acquire information on the procedural tolerability of IRE and the subsequent tumour resection and intervention-independent quality of life up to 4 months after IRE.

2.8. Study population eligibility

Men and women with imaging-based evidence of a localised, solid renal mass with suspicion of RCC ≤4 cm without any sign of metastasis (and, if applicable, kidney carcinoma confirmed by biopsy; TNM classification T1 a cN0 cM0, Stage I, EAU criteria; for an example see Fig. 1) will be considered for inclusion in the study. Imaging will be performed by contrast-enhanced CT or MRI. All histological kidney carcinoma subtypes and Fuhrman grades will be included. Selection of patients in pre-screening and screening will be performed according to the complete list of criteria in Table 1. Sample size is 20 (see Section 2.17).

2.9. Trial entry

All patients with a new diagnosis of suspected localised kidney tumour or RCC will be counselled about the standard treatment option with curative intention (partial kidney resection/tumour nephrectomy). Those meeting all the inclusion and none of the exclusion criteria (see Table 1) will be offered a Patient’s Information Sheet and invited to attend a screening and consent visit if they are interested in study participation.

Table 1

<table>
<thead>
<tr>
<th>Inclusion and exclusion criteria.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inclusion criteria:</strong></td>
</tr>
<tr>
<td>— One or more localised, resectable kidney tumours (≤4 cm) with suspicion of malignancy OR histologically confirmed renal cell cancer,</td>
</tr>
<tr>
<td>— Patient’s desire for non-surgical and surgical therapy,</td>
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<tr>
<td>— Karnofsky Index &gt; 70% and ECOG ≤ 1,</td>
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<tr>
<td>— Age ≥ 18 years,</td>
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<tr>
<td>— Life expectancy ≥ 12 months,</td>
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<tr>
<td>— Compliance of the patient taking part in a study,</td>
</tr>
<tr>
<td>— Informed consent.</td>
</tr>
<tr>
<td><strong>Exclusion criteria:</strong></td>
</tr>
<tr>
<td>— Cardiac pacemaker or other electrical implant(s),</td>
</tr>
<tr>
<td>— QT interval &gt; 550 ms or cardiac arrhythmias or any condition after myocardial infarction that makes an ECG-synchronisation unfeasible,</td>
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<tr>
<td>— Known cardiac ejection fraction &lt; 30% or NYHA 3–4,</td>
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<tr>
<td>— Known epilepsy,</td>
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<tr>
<td>— Second malignancy (except basal-cell carcinoma and cervical carcinoma in situ),</td>
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<tr>
<td>— Immunosuppression or HIV-positivity,</td>
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<tr>
<td>— Active infection or severe health impairment that makes taking part in a study unfeasible,</td>
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<tr>
<td>— Pregnancy or lactation,</td>
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<tr>
<td>— Metastatic disease,</td>
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<tr>
<td>— Palliative status,</td>
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<tr>
<td>— Current or completed therapy for RCC,</td>
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<tr>
<td>— Taking part in another clinical study in RCC,</td>
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<tr>
<td>— Rejection of interventional or surgical therapy by the patient,</td>
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<tr>
<td>— Circulatory instability,</td>
</tr>
<tr>
<td>— Inoperable or general contra-indications for anaesthesia, endotracheal anaesthesia and/or muscle relaxation,</td>
</tr>
<tr>
<td>— Psychiatric disorders that make taking part in a study or giving informed consent unfeasible,</td>
</tr>
<tr>
<td>— Haemorrhage, impossibility of taking a blood-thinner, untreated thrombophilia, thromboplastin time ≤ 50%, thrombocytes ≤ 50 Gpt/l, partial thromboplastin time ≥ 50 s,</td>
</tr>
<tr>
<td>— MRI incompatibility,</td>
</tr>
<tr>
<td>— Metal implants &lt; 1 cm close to the kidney/kidney tumour,</td>
</tr>
<tr>
<td>— Contraindication for biopsy and puncture of the renal tumour under CT guidance,</td>
</tr>
<tr>
<td>— Suspicion of renal pelvic tumour/urothelial carcinoma of the kidney,</td>
</tr>
<tr>
<td>— Untreated urinary retention of the kidney/hydronephrosis.</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic example of localised T1a kidney tumour/kidney cell carcinoma (blue circles) suitable for IRE. Kidney parenchyma (reddish brown) and renal pelvis (brown).
participation (see Fig. 2, Table 2). Central to the patient’s information therapy decision will be the tissue-sparing, kidney-preserving removal of the tumour. The study rationale, the basics of IRE and the current state of scientific knowledge will be discussed with the patient, and possible advantages and disadvantages of participating in the study will be explained.

2.10. Trial flow

See Fig. 2.

2.11. Single visit schedule

See Table 2.

2.12. Interventions

2.12.1. Pre-treatment biopsy

Percutaneous biopsy is obligatory before ablation of renal masses [4], in order to obtain an initial histological tumour classification in cases of complete ablation. If not performed previously, the IRE intervention starts with a percutaneous full-core biopsy (two-cylinder) guided by CT and/or MRI–sonography under general anaesthesia. Immediately thereafter the IRE electrodes are placed and IRE is performed. Biopsy specimens will be analysed histologically according to current guidelines [27].

2.12.2. Focal irreversible electroporation (Intervention 1)

IRE procedure is to be performed under general anaesthesia and deep muscle paralysis (the latter only during IRE pulse application) to avoid severe muscle contractions and patient movement. Maximum muscle relaxation cannot completely prevent IRE-triggered muscle contractions. The optimum level of muscle relaxation is described as 0–1 twitches per 10 IRE pulses [28]. The complex IRE electrode placement, IRE treatment planning and intermediate graph analysis require prolonged anaesthesia. Muscle relaxation, prone position, prolonged intervention, and possible IRE-triggered muscle contraction make endotracheal anaesthesia necessary. IRE requires separate ECG synchronisation (R-wave triggering, using an external synchronisation device: AccuSync® 72, AccuSync Medical Research Corporation, Milford, CT,

Visit 1 (out-patient): First contact, pre-screening → No study participation → conventional treatment

Visit 2 (out-patient): Enrolment (informed consent and screening) → Exclusion before IRE

Visit 3 (in-patient, Day –1 and 0): Contrast-enhanced MRI, questionnaires, laboratory tests, ECG → CT-guided percutaneous renal tumour biopsy (if not conducted before screening) → CT-guided percutaneous IRE treatment → Drop-out during IRE, incomplete IRE treatment → conventional treatment

Visit 4 (out-patient, 7 days after IRE): Contrast-enhanced MRI, questionnaires, laboratory tests, ECG → Drop-out after IRE but before surgical resection → individual treatment

Visit 5 (in-patient, 1 month after IRE): Contrast-enhanced MRI, questionnaires, laboratory tests, ECG → Surgical resection → Histopathological analysis → Drop-out after completed intervention sequence (IRE and surgical resection), but before study follow-up → follow-up as by recommended guidelines

Visit 6 (out-patient, 4 months after IRE and 3 months after surgery): Follow-up: contrast-MRI, questionnaires, laboratory tests, ECG → Study participation completed (secondary outcome met) → Follow-up out of study as recommended by guidelines

Fig. 2. Trial flow chart.
USA). The optimum IRE electrode configuration and the optimum probe-pair voltage setting (V/cm) for the target zone (tumour zone plus a safety margin of 5–10 mm) \[1,4,28\] is planned by using the PC-based NanoKnife® planning software demonstration tool (ProcedureManager-2_2_0_23 for Windows, AngioDynamics®[29]). The 15 cm 19G single IRE electrodes are placed under CT guidance or MRI–sonography in accordance with the pre-interventional treatment planning. A CT scan or MRI–sonography is then performed to check the current position of the IRE electrodes relative to each other and to the adjoining tumour edge. This allows precise placing of the needle-like electrodes. On the basis of these data, IRE treatment is planned by using the NanoKnife® generator-based planning software tool (AngioDynamics®). The optimum electrode-pair voltage setting is chosen for the lesion zone. IRE electrode placement has to be adjusted, if necessary, and the above-mentioned interventional planning process is repeated. IRE pulses can be applied after the optimum electrode position has been checked by 10 test pulses per electrode pair. IRE pulses are applied consecutively for each predetermined IRE probe pair. Abnormal graphs, abnormal current warnings or pulse delivery failures necessitate modification of the interventional treatment planning and probe placement, and the IRE pulse application has then to be repeated. The number of electrodes used depends on the size and the shape of the target (Fig. 3). The device is set to deliver 70–90 pulses with a pulse length of 70–90 μs in order to achieve an electrical field of 20–50 A. This current has been shown to cause complete ablation in the target area without also causing an ablative heating effect, which is possible when the current is higher [2]. If the electric field is in the optimum range, then 80–90 pulses per pair are delivered; otherwise, the voltage is modified and treatment is repeated if necessary.

### 2.12.3. Tumour resection (Intervention 2)

Partial renal resection or radical nephrectomy (if necessary) with complete resection [4] of the tumour area/IRE area is conducted.

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#### Table 2

Schedule of visits and procedures.

<table>
<thead>
<tr>
<th>Time of event</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
<th>Visit 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Days to -29})</td>
<td>(\text{Day -1})</td>
<td>(\text{Day 0})</td>
<td>(\text{Day 1 to 2})</td>
<td>(\text{Day 7})</td>
<td>(\text{Day 27a})</td>
</tr>
<tr>
<td>Milestones</td>
<td>Preparation</td>
<td>Intervention 1 (IRE)</td>
<td>Surveillance</td>
<td>Surveillance and preparation</td>
<td>Intervention 2 (surgery)</td>
</tr>
<tr>
<td>Informed consent</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Anamnesis</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>QOL questionnaires</td>
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<td>X</td>
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<tr>
<td>Physical examination</td>
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<tr>
<td>Evaluation anaesthesia</td>
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<td>X</td>
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<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Blood and urine tests</td>
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<td>Chest CT</td>
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<tr>
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<td>Kidney/abdomen contrast-enhanced MRI</td>
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<td>X</td>
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<tr>
<td>CT-guided kidney tumour biopsy</td>
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<tr>
<td>Open kidney tumour resection (partial kidney resection)</td>
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<tr>
<td>Endotracheal general anaesthesia</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Histopathological analysis</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

\[a\] Time point: +1 to +7 days, if the patient’s general condition or other circumstances make treatment at the scheduled time point temporarily impossible.

\[b\] Time point: ±7 days, if the patient’s general condition or other circumstances make treatment at the scheduled time point temporarily impossible.

\[c\] If not already done in pre-screening.

\[d\] If medically indicated according to the current guideline of the EAU.

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**Fig 3.** This is a representative sketch of the treatment (planning) for IRE of the kidney tumour (blue circle). The left image is a transversal view of a renal MRI with a lateral view of the dorsolumbar inserted IRE probes (white arrows). The right image is a coronal view of a renal MRI with coaxial view of the dorsolumbar inserted IRE electrodes (white spots). The highlighted red and yellow spots typify the IRE energy input in whole target (tumour plus safety margin).
28 days after the IRE. The intervention is performed by open surgery (to allow better assessment of the organ and its surroundings; lumbar or transperitoneal access) under general anaesthesia. Depending upon the results of the first, interim analysis, the procedures may be adapted to a laparoscopic resection. This analysis will be performed when the first three patients have been fully assessed. To obtain first insights into the IRE ablation environment in situ in the kidney, the intervention is initially planned as an open surgery to allow better assessment of the organ and its surroundings as well as lumbar or transperitoneal access. Depending on the first interim analysis, laparoscopic nephrectomy (total or partial) may be performed. To identify the spatial position of the resectate or tumour for the resection, the resectate will be marked intraoperatively with threads, as is usual.

2.13. Investigations

2.13.1. Contrast MRI of the kidney/abdomen

The image-morphological assessment of the kidneys is performed by contrast-enhanced MRI (1.5 T scanner, gadobutrol: 0.1 ml/kg Gadovist 1.0 mmol/ml; Bayer, Leverkusen, Germany) on planned study days 1, 1–2, 7, 27 and 112, whereby the contrast medium sequences ‘arterial’, ‘portal-venous’, ‘venous’ and ‘urographic’ are carried out. The sequences to be used are shown in Table 3. The above MRI parameters were defined as ideal settings in clinical routine for kidney MRI, and in earlier animal studies we found them to be optimum parameters for MRI diagnosis before and after IRE of kidney tissue [6]. DW-MRI will be analysed by two specialised urological radiologist focusing on: ablation zone, ablation zone shape/symmetry, ablation volume, residual tumour at the ablation zone border, skip lesions within ablation zone, transition zone between ablated and normal renal or perirenal tissue, and damage to vital structures. MRI images will be stacked to render a 2D–3D reconstruction of the kidney and the IRE ablation zone within it.

2.13.2. Laboratory tests — blood and urine

The following laboratory values are to be determined in the screening and/or in the course of the study (for time points see Table 2):

- Haematological and clinico-chemical values: aspartate transaminase, alanine transaminase, lactate dehydrogenase, alkaline phosphatase, albumin, C-reactive protein, sodium, potassium, chlorine, calcium, creatinine, urea, uric acid, thyroid-stimulating hormone, free thyroid hormone T4, human β-choriogonadotrophin (for women of child-bearing age), Quick’s test, partial thromboplastin time, thrombin time, coagulation time; serology for human immuno-deficiency virus and hepatitis; standard haematological values, and blood group.
- Urine tests: sediment, bacteria (culture), and urine cytology [30].

2.13.3. Scores and questionnaires on morbidity and quality of life

The patients’ morbidity and quality of life will be recorded systematically by means of standardised scores and questionnaires [1], inter alia, the effects of the therapeutic interventions – IRE and surgery – on quality of life will be investigated. The following scores and questionnaires will be used:

- Life expectancy of the patient by age and sex as given in the mortality statistics issued by the German Statistisches Bundesamt, Wiesbaden, 2013 [31].
- Charlson Comorbidity Index/Score [32].
- Degree of pain on a numerical rating scale (NRS) from 1 to 10 according to the Ärztliches Zentrum für Qualität in der Medizin [33] in the context of a pain diary.
- Quality of life according to EORTC QLQ-C30, Version 3.0, German [34].
- Disease-specific morbidity and quality of life (kidney carcinoma) according to FKSI-15 (Functional Assessment of Cancer Therapy (FACT) Kidney Symptom Index-15), Version 4.0, German [35].
- Study-specific quality of life as assessed by using a study-specific questionnaire (questions 1–9) according to Wendler, adapted from EORTC QLC-C30, Version 3.0, German (aEORTC-QLQ-C30-Wendler).
- ECOG Score and Karnofsky Index.
- Grading of adverse events following the Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, of the U.S. NIH and following the Clavien–Dindo classification [36].
- Grading of adverse events following the SIQR (Society of Intervenional Radiology) classification system for complications by outcome [1,26,37].

2.13.4. Histopathological analysis

After resection, preparation sections are fixed in buffered 4% formaldehyde solution for at least 24 h and then examined by microscopy. This includes the complete sectioning of the ablation area including a border of macroscopically inconspicuous kidney tissue in 0.5 cm thick slices [27]. The slices are numbered and photographed for documentation. The ablation area is measured. In cases of nephrectomy, coarse sectioning of the remaining preparation to exclude other pathologies is done. Thereafter the ablation area, including the border, is completely embedded in paraffin wax in standard tissue cassettes after topographic assignment based on macrophotography. Each tissue block is used to prepare 3 μm thick sections and these are stained with haematoxylin–eosin (the number of sections depends on the thickness of the tissue) for morphological assessment in 500 μm steps with one unstained section each.
for transmission microscopy. Additional immunohistological staining (e.g., Mib-1) is performed to determine viability or irreversible cell death [1]. Further detailed analysis includes the measurement, mapping and photo-documentation of the histological preparation by transmission microscopy.

The histological work-up will be conducted in the marked plane with corresponding mapping as 2D fusion sketches and possibly 3D reconstruction. In each specimen, the complete ablation area, the tumour area and non-affected renal tissue on each slide will be precisely outlined in multicoloured ink. On the basis of this marking, sequential outline maps will be traced for each level of section. The maps from each subject will be computer-scanned and inspected serially. The area of tumour at each level of section will be determined by using a digitising pad (Bamboo One™) and image-manipulation software (GIMP 2.8.14®). This reconstruction will be used to assess the exact lesion volume (volumetry) and shape.

2.14. Observation and follow-up

The total period of observation and follow-up is 16 weeks (approximately 4 months), i.e., 16 weeks after IRE and 12 weeks after surgical resection. After this, the patient’s participation in the study will be complete. At the final study follow-up examination (16 weeks after IRE) the following measurements and assessments will be performed: general clinical examination, body weight and height, vital signs (blood pressure, pulse, temperature), general health status, recording of quality of life and morbidity (by ECOG status, Karnofsky Index, FACT-FKSI-15-Dindo and the SIR classification), and three-phase contrast-enhanced MRI (high-field MRI, 1.5 T) of the kidneys.

Thereafter, continued care, on an individual basis and within the responsibility of the patient’s own urologist, is recommended. This should follow the EAU guidelines [4] and may involve referral back to the study centre; it should include CT of the thorax and abdomen as practised for example, in the context of the intended kidney tissue (collateral damage) in the context of the intended kidney tissue (collateral damage).

2.15. Management of serious adverse events

For all patients in this clinical trial, all adverse events after the giving of informed consent will be recorded in a GCP-compliant manner and followed up as necessary. Lack of effectiveness of the study treatment will not be regarded as an adverse event.

Serious adverse events (SAEs) are those that fulfill at least one of the following criteria (note that the list is based upon those set out in GCP, but goes beyond them in the inclusion of certain procedure-related events). SAEs are listed in Table 4 Appendix.

Every SAE will be assessed by the principal investigator, in order to allow the continual evaluation of the risk–benefit ratio of the study treatment and to ensure the patient’s safety during the subsequent course of the trial.

The trial’s Steering Committee, the principal investigator and the sponsor (the Clinical Study Centre of the Medical Faculty of the OVGU) are legally obliged to report all SAEs and all adverse events leading to discontinuation of a patient’s participation in the study to the BfArM (as responsible regulatory authority) and to the Ethics Committee of the Medical Faculty of the OVGU.

2.16. Stopping rules

The following events are to be regarded as sufficient grounds for withdrawing individual patients from the study:

- Personal wish of the patient;
- Decision of the principal investigator, if in his considered view the patient is likely to profit from a change in treatment according to the EAU guidelines [4];
- Decision of the sponsor, for major regulatory or ethical reasons;
- If in the view of the investigation team the risks for the patient outweigh the benefits;
- If the recruitment of patients is clearly delayed for objective reasons.

The reasons for premature withdrawal of a patient from the trial are to be documented in the source data (patient’s records) by the investigator and, within the framework of SAE management, to be reported to the Clinical Study Centre of the Medical Faculty of the OVGU. After every SAE report the trial’s Steering Committee and sponsor (the latter usually represented by the Clinical Study Centre of the Medical Faculty of the OVGU) will assess whether it is safe to proceed with the trial. After every SAE report the BfArM will assess whether it is safe to continue the study and other studies or to allow the conduct of other studies – and treatment outside clinical studies – with the Nanoknife Electroporation Ablation System. After every SAE report Ethics Committee of the Medical Faculty of the OVGU will issue a recommendation as to whether it is ethically justified to continue the study.

2.17. Statistical analysis and sample-size calculation

On the basis of the aims and goals and the primary and secondary objectives of this Phase 2a pilot study the sample size was set at N = 20 [39,41,43]. This cohort size was chosen by the sponsor on the basis of experience with similar studies and was not based upon a formal calculation. It was agreed to by the BfArM and the Ethics Committee of the Medical Faculty of the OVGU. This cohort size will allow meaningful assessment of technical feasibility and of the success or failure of the ablation, by histological and imaging criteria. Precedence is here given to obtaining a detailed descriptive evaluation, without significance testing, for which the study would in any case presumably be underpowered. Statistical analysis will be performed by a study statistician of the medical faculty of the OVGU with IBM SPSS Statistics (SPSS; version 22). This pilot study is intended to provide the basis for a confirmatory, multicentric trial.

2.17.1. Primary outcomes

Primary outcomes will be measured by conventional histopathological analysis and special proliferation markers for formalin-fixed paraffin-embedded specimens to determine the success of the tumour ablation and to detect any persisting viable tumour tissue.

2.17.2. Secondary outcomes

Secondary outcomes will be measured by conventional histopathological analysis and by kidney imaging by diffusion-weighted contrast-enhanced MRI for the determination of damage to healthy peritumoural kidney tissue (collateral damage) in the context of the intended nephron-sparing effect. Furthermore, the tolerability of the procedure and the intervention-dependent quality of life following IRE and the subsequent tumour resection will be assessed on the basis of quality-of-life questionnaires (EORTC QLQ-C30, V3.0D, FKSI-15-V4.0D, adapted EORTC QLQ-C30 V3.0D (aEORTC-QLQ-C30-Wendler)), ECOG status, Karnofsky Index) and the NRS, and by classification of adverse events according to CTCAE version 4.0, the Clavien–Dindo and the SIR classifications.
In the context of the complete documentation of the procedure and adverse events, the trial is aimed at assessing the technical feasibility of the percutaneous IRE, supported by CT and/or MRI–sonography, of localised kidney tumours using the commercially available NanoKnife Electroporation Ablation System under study conditions close to those of clinical practice.

2.18. Monitoring and audit

Regular monitoring will be performed by the external and independent Co-ordinating Centre for Clinical Trials of the Medical Faculty of the Martin Luther University of Halle-Wittenberg (KKS Halle), which is a member of the German network of co-ordination centres for clinical studies (KKS network). Audits will be regularly performed by independent staff of the Clinical Study Centre of the Medical Faculty of the OVGU. The purpose of the monitoring and the audits is to contribute to ensuring patient safety and adherence to GCP.

2.19. Legal and ethical principles

The study will be conducted in conformity with the most recent version of the Declaration of Helsinki (October 2013). It will adhere to GCP (German version, "Good Clinical Practice" — Verordnung; GCP-V, most recent version, of 19.10.2012); Grundsätze für die ordnungsgemäße Durchführung der klinischen Prüfung von Medizinprodukten ("Principles of good practice in the conduct of clinical trials of medicinal products", Bundesanzeiger, DIMDI), the German law governing medicinal products (MPG; most recent version, 21.07.2014), the European norm for the application of risk management for medicinal products (Norm EN ISO 14971:2012 according to the European guideline 93/42/EWG) and international operating procedures for medicinal products (ISO 14155:2011).

3. Discussion

Following on from pre-clinical studies testing IRE in phantoms (immediate evaluation), ex vivo porcine kidney (immediate evaluation), in vivo porcine kidney (short- and medium-term evaluation) and human kidney tumours in situ (immediate evaluation; Phase 1 safety study), the prospective Phase 2a trial IRENE represents the natural next step in the assessment of this new technology for treating localised renal cell cancer [38]. The IRE procedure and the NanoKnife offer a number of user-dependent variables, so that the parameters of IRE treatment can be specified exactly and used to compare various study results in evaluating the effectiveness and safety of the treatment and the results obtained from imaging and histology [1]. Authors should also set out to describe the tissue homogeneity of the target tumours and to stratify/quantify any inhomogeneity in tumour-specific tissue and in organ structures [1]. Post-procedural imaging findings are only a rough guide to the success of ablation therapy, because microscopic foci of residual disease cannot be expected to be identified by standard imaging techniques [1,26]. Absence of – or a minimal involution of – the ablation zone does not imply treatment failure [1,26]. Any residual tumour tissue or tumour cells should result in the result being classified as incomplete ablation [1], independently of how small the amount of residual tumour may be in relation to the untreated tumour. IRE may be reversible or irreversible, depending on the voltage applied and the peripheral decrease in field strength (edge area), whereby however only the irreversible region leads to visible tissue ablation [5]. One of the short-comings of IRE today is the absence of dedicated, clinically validated protocols to be used in different tissue environments and for different tumour biologies [39,40]. In addition, no technical endpoint has yet been described that reliably predicts successful ablation of a tumour during the course of the intervention procedure [1,39]. So far, there is a lack of adequate experience of how long it takes for complete tumour involution to occur after correctly performed ablation by IRE, or of how long after IRE one may expect to observe residual tumour cells if the ablation was incomplete. Preliminary studies in renal IRE suggested the 4-week interval to be used in the present study, without however, having been conducted on a tumour model. It is possible that tumour tissue be- haves differently from healthy kidney parenchyma. It is also unclear whether residual nests of tumour cells in coagulation necrosis of the ablation zone can influence the oncological outcome. For that reason a secondary objective of the IRENE study is also defined as “probable oncotherapeutic effectiveness” measured by the proportion of residual viable tumour in the kidney, as revealed by histopathology and imaging 28 days after IRE” (see Aim of the study). It is to be expected that post-interventional, reversible effects of IRE in the period after 28 days will no longer be detectable, either by imaging or by histology. The monitoring by MRI and post-interventional biopsy probably does not provide adequate proof of the success of therapy. For this reason the study includes a complete resection of the tumour – or ablation area – with a complete histological work-up. The oncotherapeutic security of the patient is guaranteed by the inclusion of standard, guideline-compliant tumour resection. IRE is a procedure with potentially attractive characteristics and may be ideal for delivering focal therapy in the kidney, especially as it has been shown to have tissue-selectivity in animal studies that have demonstrated a homogeneous ablation of renal tissue with preservation of larger vessels and the urine-collecting system [6,11,12,14,15].

To our knowledge, there is no description of needle-tract tumour seeding after coaxial biopsy and thermal RCC ablation by RFa or cryo-therapy [4]. Needle-tract tumour seeding was first described for IRE in the context of IRE ablation of lung tumours [39]. However, in the context of IRE of kidney tumours this has not been observed [13,17]. Although the design includes repeated MR imaging before and after IRE, MRI seems to be the ideal but not validated imaging tool for local monitoring after IRE [6]. As a consequence of these considerations, the only reliable measure of ablation in this study remains the histological analysis of the completely resected tissue in the treatment area.

3.0.1. Study limitations

The study is a single-centre trial in a tertiary university hospital that has especial expertise in focal therapy, image-guided therapy and minimally invasive percutaneous kidney procedures; therefore, the results of this study are unlikely to allow wide generalisation. Furthermore, the small cohort size (20 patients) will clearly leave a need for future multi-centre studies. This single-centre expert setting is the preferred context to allow the development of new technologies and treatment procedures such as percutaneous image-guided renal IRE. At present, a technical limitation of IRE by the NanoKnife Electroporation Ablation System is the absence of any real-time monitoring of the energy applied or of therapeutic success; to date no clinically applicable solution to meet this need was been found. The position of the kidney tumour does not constitute a criterion for participation in the IRENE trial. Therefore, systematic measurement errors due to the technically imposed limits on slice thickness have to be taken into account. In the assessment of quality of life, some of the questionnaires to be used record only approximatively, on a “best possible” basis, in the absence of validated alternatives, the patient’s actual life quality in the context of his/her localised kidney tumour or carcinoma and its ablation. Differences in tumour biology and response rate of different histological entities (RCC vs. transitional cell cancer or renal metastasis) must be taken into account in any consideration of the generalisability of the study’s results. The possible disadvantages of participation in the study vis-à-vis standard tumour-resection therapy are puncture and IRE as additional invasive procedures under general anaesthesia. In CT/fluoroscopy-supported IRE electrode placement there is also additional exposure to X-rays. Furthermore, with 4 weeks an especially short interval was chosen, so as to avoid any prognostically relevant delay of the definitive therapy (R0 resection, gold standard). On account of the postulated massive tumour damage by IRE, the risk of a secondary carry-over of tumour cells...
4. Conclusion

The IRENE trial will allow an appropriate evaluation in a clinical setting of the feasibility and effectiveness of CT-guided and/or MRI-sonography-guided percutaneous IRE ablation of localised renal cell cancer as well as functional outcomes, using pre- and post-treatment MRI and ultra-thin-section histology after complete surgical resection in the short-term interval. The outcomes of the IRENE trial may be used to plan a larger multi-centre confirmation study. Our pilot study protocol of renal IRE as a potential universal tumour model can provide guidance for designing an effectiveness and feasibility trial to assess a new ablative technology in the challenging landscape of surgical and focal treatment particularly for RCC. A different tumour biology and ablation response of the different tumour entities respectively RCC subtypes have to be taken into account and discussed.

Conflict of interest

M. Schostak has received funding for conference attendance from AngioDynamics Inc. (New York, USA). J.J. Wendler has received trial funding support from AngioDynamics Inc. for previous experimental studies. Neither of these sources provided any input whatsoever into this article or this study.

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Appendix A

Table 4

<table>
<thead>
<tr>
<th>SAE definition</th>
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<tr>
<td>Death during the study or up to 28 calendar days after the ending of study-specific procedures. (Death because of confirmed tumour progression will not be recorded as an SAE and will be recorded in the CRF with the clinical cause of death documented as an adverse event, severity “5 = death”. An SAE report for this is not required.)</td>
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<tr>
<td>The adverse event is immediately life-threatening.</td>
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<td>The adverse event leads to unplanned hospitalisation.</td>
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<td>A stationary hospital stay is extended by ≥2 days (after IRE ≥ 3 days; after resection of the kidney tumour; ≥12 days) beyond the usual duration of confinement to bed.</td>
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<tr>
<td>Persistent and/or interventions-requiring cardiac rhythm disorder leading to arrest of the IRE or occurring after the IRE.</td>
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<tr>
<td>Circulatory dysregulation leading to arrest of the IRE.</td>
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<tr>
<td>Epileptic attack after IRE.</td>
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<tr>
<td>Movement of the IRE electrodes, caused by muscle contraction, with injury of non-target organs or non-target tissue during or after IRE.</td>
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<tr>
<td>Retroperitoneal, perirenal abscess.</td>
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<tr>
<td>Postrupture, symptomatic, retroperitoneal haematoma requiring intervention and/or a symptomatic haemoglobin decrease after puncture, requiring intervention (definitions of indication for transfusion according to transfusion guidelines).</td>
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<td>Injury to neighbouring organs caused by puncture.</td>
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References


