Focal therapy in prostate cancer

van den Bos, W.

Citation for published version (APA):

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FOCAL THERAPY IN PROSTATE CANCER

Willemien van den Bos
The research described in this thesis was performed at the department of Urology in collaboration with the department of Biomedical Engineering and Physics, Academic Medical Center, Amsterdam, the Netherlands.

The research was funded by the Cure for Cancer Foundation. Printing of this thesis was supported by financial contributions of the Cure for Cancer Foundation, AngioDynamics, AbbVie B.V., Astellas, Astra Zeneca, Blue Ribbon, Chipsoft B.V., Ferring B.V., Olympus Nederland B.V., St. Jude Medical Nederland B.V. and Academic Medical Center.

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FOCAL THERAPY IN PROSTATE CANCER

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom
ten overstaan van een door het College voor Promoties
ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op donderdag 26 mei 2016, te 10.00 uur

door

Willemien van den Bos
genboren te Apeldoorn
## PROMOTIECOMMISSIE

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Faculteit der Geneeskunde
Voor mijn ouders
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Supplements

Appendix; Author contributions; AMC Graduate School PhD portfolio; List of publications; Curriculum vitae; Dankwoord
Chapter 1

General introduction

This chapter provides a general background on prostate cancer and focal therapy in prostate cancer. It gives an overview of the different focal therapy techniques, the applications and outcomes.

Parts of the chapter are based on book chapter 10 in Pelvic Cancer Surgery: Minimally invasive therapies for pelvic urological cancer, Springer London 2015 by W van den Bos, BG Muller, DM de Bruin, JJMCH de la Rosette
BACKGROUND

Prostate cancer

Epidemiology

Prostate cancer is the most common malignancy in men in the Western world with the highest incidence of > 200 per 100,000 in North-western Europe [1]. Approximately one in every six men will be diagnosed with prostate cancer during their lifetime. The incidence rates have been increasing the last decades, mainly due to the intensified use of prostate-specific antigen (PSA) tests whereas PSA screening, improved prostate biopsy techniques and better-quality imaging technologies [2]. This has led to stage and grade migration towards diagnosis of less high-risk prostate cancers confined within the borders of the prostate without the evidence of extraprostatic disease or metastases. In 80% of men with prostate cancer, a clinically localized stage is identified at diagnosis. Prostate cancer is commonly suspected on the basis of digital rectal examination (DRE) and raised prostate-specific antigen (PSA) levels and is usually histopathologically confirmed by transrectal ultrasound (TRUS)-guided biopsy.

Treatment

Current treatment options for prostate cancer include active surveillance and radical whole-gland destruction through radical prostatectomy or radiation (external beam or brachytherapy). Active surveillance is indicated for patients diagnosed with low-risk prostate cancer and it has the obvious advantage of having no physical side effects. Hence the opportunity to treat the cancer while it is curable may be missed. The radical treatments are associated with well-documented morbidity including urinary incontinence, erectile dysfunction and bowel toxicity. Focal therapy is therefore gaining interest offering the middle ground between active surveillance and radical treatment. The challenge of current focal therapy techniques is to treat the tumorous tissue while sparing the benign parts of the prostate and surrounding tissue, in order to minimize the side effects.

Focal therapy

A variety of ablation techniques have been introduced for the treatment of localised prostate cancer. These techniques include brachytherapy, cryotherapy, high-intensity focused ultrasound (HIFU), laser ablation therapy, radiofrequency ablation, irreversible electroporation (IRE) and photodynamic therapy (PDT). The first three modalities have emerged as alternative therapeutic options in patients with clinically localised prostate cancer not suitable for radical prostatectomy, by the European Urology Association.
and American Urological Association. The others are still considered experimental [3]. Because of increased detection of early stage local prostate cancer lesions, focal therapy fulfills a more significant role as a less invasive procedure in the management of the disease [4]. The patients with high volume low risk and intermediate risk prostate cancer with localized disease are the best candidates for focal treatment. Especially when it contains unilateral disease and clinical stage ≤cT2a. [5] (More details in Table 1)

**Table 1. Ideal candidates for focal therapy**

<table>
<thead>
<tr>
<th>Serum PSA</th>
<th>PSA &lt; 15 ng/mL, PSA &gt; 15 ng/mL should be counselled with caution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical stage</td>
<td>T1c-T2a</td>
</tr>
<tr>
<td>Pathology</td>
<td>Gleason score 3+3</td>
</tr>
<tr>
<td></td>
<td>Gleason score 3 + 4</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>&gt; 10 years</td>
</tr>
<tr>
<td>Volume</td>
<td>Any; except in case of HIFU: &lt; 40 mL</td>
</tr>
</tbody>
</table>

Focal treatment enables better tissue preservation, decreased morbidity and is potentially applicable in 50–66% of men with prostate cancer [6; 7]. The different treatment scenarios are ultra-focal, hemi-ablation or whole-gland therapy, based on the localisation and multi-focality of the tumours. The minimally invasive nature of these techniques usually results in a short hospital stay with a better side effect profile and less impact on quality of life, resulting in increased popularity [8; 9]. Nevertheless, since the available diagnostic modalities are still not yet conclusive, it is important to take into account that small insignificant lesions can be kept undetected and therefore untreated. In this chapter, the principles of each focal modality as well as information on application and outcome are provided to help understanding the different techniques.

**Thermal ablations**

**Laser ablation therapy**

**Principles**

Laser ablation therapy uses near infrared (NIR) light from a neodymium-yttrium-aluminum-garnet laser. It reaches the tissue of the prostate by laser fibres through a transperineal approach. The technique is based on the photo-thermal effect. This thermal action results from the absorption of NIR light by tissue chromophores, which is converted into heat in a very short time [10]. This effect depends on the intensity of light and the concentration of available tissue chromophores. Temperatures above 60 °C cause rapid coagulative necrosis in the targeted tissue followed by instant cell death. But also at lower hyperthermic temperatures (>42°C) irreversible cell death is also achieved with prolongation of the procedure [11; 12].
Application and outcome

Besides the destruction of cells by the photo-thermal effect, a reaction can be observed in the reduction of blood perfusion. It is therefore possible to observe the delineation between viable and nonviable tissue with contrast-enhanced ultrasonography (CEUS) [13]. Until now, all studies about laser ablation are phase I clinical trials or contain small cohorts with a maximum of 12 patients [14]. This study of Lindner et al. showed on biopsies after six months post-treatment 67% was free of tumour in the targeted area and 50% was free of disease. Side effects according to this technique included perineal discomfort, haematospermia, dysuria and fatigue [13-15]. Further research is needed to demonstrate the long-term effectiveness.

Cryotherapy

Principles

Cryotherapy induces cell death by freezing. In the past, urinary incontinence, urethral sloughing and recto-urethral fistula were common side effects and a mortality rate of 1.9% was reported [16]. More recently, the technique has changed and improved using multiprobe-devices, guided by advanced imaging techniques. Cryotherapy is either used as primary treatment (partial or whole-gland) or as salvage treatment. It contains different mechanisms in destroying tumour tissue, including ‘Freeze rupture’, a cellular responses to freezing, which induces cell death known as necrosis and apoptosis [17]. Direct cell damage occurs when cell metabolism fails as a result of temperature drop. When temperature decreases until -20°C, extracellular water crystallizes and causes a retraction of water out of the system. This results in a hyperosmotic extracellular environment followed by the extraction of water from the cells and end up in denaturation and electrolyte disturbances [18]. All parts of the freeze–thaw cycle can cause tissue damage. But the coldest tissue temperature is the main factor in generating cell death. It is also important that the cooling rate is as fast as possible. The optimal duration of freezing are unknown, but long lasting freezing increases tissue injury. Thawing rate is a prime destructive cause and it should be as slow as possible, thereby repetition of the freeze–thaw cycle is an important factor in effective therapy [19]. Furthermore, freezing until at least -40°C is recommended since this causes intracellular ice crystal formation which is a severe threat to cell viability and nearly always lethal [20:21]. Depending on the multifocality and extension of the tumour, the choice has to be made between whole or partial-gland cryoablation.

Application and outcome

Cryotherapy is an option for low-, intermediate-, and high-risk patients [17]. The freezing is obtained by introducing TRUS -guided needles using a transperineal approach. Limiting factor is the volume of the gland; the larger the prostate, the
more difficult to achieve a uniformly cold temperature by pubic arch interference. Advantage is the ability of real-time visualisation of the formed ice-ball by TRUS or Magnetic Resonance Imaging (MRI). Short-term complications are urinary retention because of gland swelling. Penile and scrotal swelling can occur, but are mostly self-limiting. Long-term morbidity differs between partial-gland and whole-gland treatment. A report from the National Cryo On-Line Database (COLD) Registry shows a high percentage of complete urinary continence (98.4%) [22]. Erectile dysfunction ranged from 49 percent to 93 percent at one year [23;24]. Here for, cryoablation is considered as a treatment option in men who are not concerned with erectile function. Biochemical disease-free survival is diversely ranged along patients-cohorts. The 5-year biochemical disease-free survival rates for low-, intermediate-., and high-risk cases range from 65 percent to 92 percent, 69 percent to 89 percent, and 48 percent to 89 percent, respectively [17;24]. Another study (n=60) shows biopsy proven recurrence found in up to 23% of the patients after 15.2 months, mostly found in the untreated hemi-gland [25].

**High-intensity focused ultrasound**

**Principles**

High-intensity focused ultrasound (HIFU) has seen several applications in tissue since the fifties. Madersbacher et al [26] stated in 1995 the value of HIFU in treating prostate cancer, leading to several clinical studies. HIFU uses focused ultrasound (US) to destroy tissue based on two principles; hyperthermia and cavitation. When the US beam is focused transrectally at a specific depth inside the prostate, the high-energy instigates heat above the denaturation-temperature of proteins inducing cell death. Besides, the US beam can interact with aqueous micro-bubbles in the sonicated area, leading to coagulative necrosis [27-29]. Two companies are providing HIFU devices: Ablatherm and Sonablate.

**Application and outcome**

HIFU is used as well as primary treatment as salvage treatment. Best candidates for HIFU are patients with T1c-T3 tumours smaller than 40 mL that are not suitable for radical approach. Contra-indication is the absence or an inaccessible rectum since the technique applies a transrectal approach. Also, major calcifications larger than 1.0 cm have negative influence on treatment [30;31]. The advantage of this procedure over other focal therapies is the ability to destroy cells over a distance from the US probe without being invasive. Most common complications of HIFU therapy are urinary retention (<1-20%) caused by oedematous prostate tissue, urinary tract infections (1.8-
Erectile dysfunction is reported in 20.0 to 81.6%, which is less compared to other modalities. The incidence of recto-urethral fistulas (<2%) has decreased with the improvement of devices and treatment procedures. Most complications are transient or treatable. Less common complications are urethral or bladder neck stenosis, urethral strictures, chronic perineal pain, infravesical obstruction, epididymitis, and prostatitis [32;33]. Cordeiro et al [32] reviewed the outcome of 31 HIFU studies and stated that negative biopsy rates (mostly taken after 3-6 months) ranged from 35 to 95%. Percentage of patients with a PSA nadir of 0.5 ng/mL ranged from 61-91%. The 5-year biochemical disease-free rate (according to Phoenix criteria) was 72%; 84% for low-risk patients, 64% for intermediate-risk patients and 45% for high-risk patients [31].

Radiofrequency ablation
Principles
This technique uses radiofrequency energy to ablate tissue. Through transperineal needles the monopolar electrodes can be inserted which are able to reach 50 W with a frequency of 460 kHz. It causes an irreversible destruction of tissue by hyperthermia of approximately 100°C. Hyperthermia occurs by gradually raising the power. For five minutes this heat has to be maintained. This results in coagulative necrosis of the targeted tissue [34]. During this treatment, the urethra and rectum were cooled by cold saline. The procedure has been assessed as feasible, safe and reproducible in prostate cancer [35;36].

Application and outcome
Radiofrequency ablation has been investigated in two different groups of patients. Firstly in patients with clinically localized prostate cancer; this showed no complications [35;36]. Shariat et al. [37] treated patients after failed radiation and patients unfit for surgery. This study showed transient side effects as macrohaimaturia in 19%, bladder spasms and dysuria in 9%. At 12 months after RITA, 50% of patients with sufficient follow-up had no residual cancer on repeat systematic 12-core biopsy cores and 67% were cancer-free in biopsy cores sampled from the RITA-treated areas. No long-term outcomes have been reported in literature.
Non-thermal ablations

Irreversible electroporation

Principles

Bio-electrics are an interesting new area of medicine combining pulsed high-voltage engineering and cell biology [38-40]. Pulsating current alters the transmembrane potential of biological cells. If the duration of the applied electrical pulses is below the charging time of the outer cell membrane (approximately 100 nanoseconds for mammalian cells), there is non-thermal interaction of the electric field with subcellular structures. Cell survival is inversely proportional to the electric field generated and by manipulating the pulse duration, the electric field intensity, and the number of pulses, it is possible to alter the effects on the target cells. The pulsed electric fields increase the permeability of the cell membrane by a process known as electroporation, a process that can be reversible or irreversible depending on the combination of the variables above [41]. Reversible electroporation temporarily makes the cell membrane more permeable [42]. The cell can survive this insult [43], and it has been employed in electro chemotherapy, to facilitate the uptake of chemotherapeutic agents into cells, and gene therapy [44;45]. Irreversible electroporation results in the permanent permeabilization of the cell membrane, which disrupts cell homeostasis and leads to cell death [42;43]. In vitro, it has commercial application and has been used by the food industry to sterilize and pre-process food since 1961 [41]; it can also be used to sterilize water, because the process destroys bacteria and yeasts. In vivo, the irreversibly permeabilized cells are left in situ and are removed by the immune system [45].

Applications and outcome

IRE has been shown to effectively ablate tumour cells in vitro, in small and large animal experiments [43;46;47] and in a recent safety study on the IRE of focal liver, kidney and lung tumours [48;49]. There are two main factors driving research into IRE as a treatment modality. First, tumour ablation experiments in animals and humans have shown that connective tissue structure is preserved and there is no damage to associated blood vessels, neural tissue, or other vital structures [43;47;50]. Second, since IRE is in theory not thermally based there is no “heat sink” effect, a factor that decreases the effectiveness of other ablation therapies such as HIFU and RFA near major vessels [45;51-53]. It is anticipated that the preservation of surrounding tissue will reduce treatment-induced side effects inherent in current prostate cancer therapies.
**Brachytherapy**

**Principles**
Brachytherapy is broadly used in the management of localised prostate cancer. Brachytherapy is the delivery of radiation by radionuclides using sealed sources, placed close to the target. Guided by TRUS, hollow needles are placed inside the prostate. Radioactive seeds are injected through these needles for permanent implantation. This precise source placement enables high dose delivery within the tumour, avoiding structures as urethra, neurovascular bundle or rectum to be irradiated and accurate doses at the margins [54;55].

**Application and outcome**
Brachytherapy can be considered in patients with clinically localized stage 1-2 prostate cancer without metastases. The average overall-survival after whole-gland brachytherapy is equivalent to options as active surveillance, radical prostatectomy (RP) or external beam radiotherapy (EBRT). Therefore, the patients have to be given the choice of treatment [56]. Biggest advantage of brachytherapy is the minor interruption of daily life of the patient with a hospital-stay of 1-2 days. Recently focal application of brachytherapy has been investigated in treatment of prostate cancer. Until now only primary outcome parameter is adverse events because of the short follow-up time [57].

The most common side effect is urethritis, which is treated with alpha-blockers and non-steroidal anti-inflammatory drugs. Also, proctitis occurs frequently. Therefore, antibiotic prophylaxis is regularly prescribed after implantation [58]. After the procedure, about 15% of the patients develop temporary acute urinary retention due to oedema, which can be solved by catheterization. Fifteen-year biochemical control is 85.9%, 79.9%, and 62.2% for low, intermediate, and high-risk patients, respectively treated with whole-gland brachytherapy [59]. Two focal brachytherapy studies are recently undertaken, concerning hemi-gland and ultra-focal procedures with the most important parameter of occurred adverse events [57;60]. A consensus has been made about patient selection for ultra-focal brachytherapy by Langley et al.[61]

**Photodynamic therapy**

**Principles**
Photodynamic therapy (PDT) was initially described at the start of the 20th century [62;63]. The technology is based on the interaction of a photosensitive agent (PS), which is administered systemically (intravenously or orally), with light brought to the tissue by a laser fibre, and oxygen that is present in the tissue. The absorption of
a photon leads to a chain reaction inducing the release of a singlet oxygen and antioxidant enzymes. This singlet oxygen can directly kill tumour cells by induction of necrosis and apoptosis, or cause destruction of tumour vasculature, producing an acute inflammatory response that attracts leucocytes, such as dendritic cells and neutrophils [64]. Accomplishment of PDT requires intraprostatic laser fibre placement. This is achieved through transperineal approach using a brachytherapy template under TRUS guidance. After fibre placement, interstitial illumination must be conducted in a darkened room to prevent cutaneous photosensitation.

**Application and outcome**

Arumainayagan et al. and Azzouzi et al. recently presented two studies including 40 and 85 patients [65;66]. In both Padeliporfin was used as photosensitizer. Arumainayagan et al. performed hemi-ablation and near whole gland with transperineal approach. MRI showed visible necrosis areas and only in two patients a side effect (recatherization) occurred. Azzouzi et al. achieved hemi-ablations that led to 87% necrosis in the treated lobe. Side effects as prostatitis, haematuria and strictures occurred. Until now, no articles are published with data on biochemical control or other outcomes. Table 2 gives a schematic overview of the different techniques with indications, contra-indications, advantages and disadvantages.
### Table 2. Overview of focal therapies

<table>
<thead>
<tr>
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<th>Contra-indications</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
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<td>Stage T1b to T2a</td>
<td>Recent transurethral resection of prostate</td>
<td>Outcomes equal to radical approach</td>
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<td>High IPSS</td>
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<td>Worse flowmetry</td>
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<td>Prostate volume &gt; 50 mL</td>
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<td></td>
<td></td>
<td>Previous pelvic irradiation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prostate volume &gt; 40 mL</td>
<td>Real time monitoring with TRUS and MRI</td>
<td>Cold sink effect</td>
</tr>
<tr>
<td>Cryotherapy</td>
<td>Stage T1 to T3 Unfit for surgery</td>
<td>No intraprostatic needles required</td>
<td>Heat sink effect</td>
</tr>
<tr>
<td></td>
<td>Life expectancy &lt; 10 years</td>
<td>Short hospital stay</td>
<td>High post-operatively impotence</td>
</tr>
<tr>
<td></td>
<td>Prostate volume &gt; 40 mL</td>
<td>Minimal rectal injury</td>
<td>Intraprostatic needles insertion required</td>
</tr>
<tr>
<td></td>
<td>Anterior tumour or tumour located near apex or midline</td>
<td></td>
<td>High costs</td>
</tr>
<tr>
<td>HIFU</td>
<td>Stage T1-T2</td>
<td>Can be performed with IV sedation in an outpatient setting</td>
<td>Heat sink effect</td>
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<td>Clinically localized cancer; not further specified</td>
<td></td>
<td>Few data about efficacy</td>
</tr>
<tr>
<td>Radiofrequency ablation</td>
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<td>Not described</td>
<td>Heat sink effect</td>
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<td>Laser ablation therapy</td>
<td>Clinically localized cancer; not further specified</td>
<td>Not described</td>
<td>Few data about efficacy</td>
</tr>
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<td></td>
<td>MRI-guidance possible</td>
<td>MRI-guidance possible</td>
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<td></td>
<td>Erectile function preservation</td>
<td>Erectile function preservation</td>
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<td></td>
<td>Short hospital stay</td>
<td>Short hospital stay</td>
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<td></td>
</tr>
<tr>
<td>Irreversible electroporation</td>
<td>Clinically localized cancer; not further specified</td>
<td>No heat sink issues</td>
<td>Intraprostatic electrodes required</td>
</tr>
<tr>
<td></td>
<td>Not described</td>
<td>Real-time CT/US imaging</td>
<td>No data about efficacy</td>
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<td>Nerves and vessel-sparing</td>
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<tr>
<td>Photodynamic therapy</td>
<td>Clinically localized cancer; not further specified</td>
<td>Photosensitizer possible selective for malignant cells.</td>
<td>Intraprostatic fibres</td>
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<td></td>
<td>Not described</td>
<td>Short hospital stay</td>
<td>Oxygen-dependency in hypoxic tumours</td>
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<td></td>
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<td></td>
<td>Technique only studied as salvage therapy</td>
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AIM OF THESIS

The aim of the studies described in this thesis is advancing the field of focal therapy in prostate cancer. To that end, research results are presented on recommendations for standardized trial design followed by early in-vivo results of irreversible electroporation of the prostate.

OUTLINE OF THIS THESIS

This thesis consists of ten chapters. Chapter 2 describes a multistage Delphi process agreement on patient selection, pre-treatment assessment, evaluation of outcome, and follow-up. This consensus report provides a standard for focal therapy trial design, which is considered important because clinical trials have been conducted with such different design characteristics that the outcomes are regarded as scarcely comparable. The recommendations were made in consultation with a multidisciplinary board from oncologic centres worldwide.

Irreversible electroporation relies on high voltage low energy direct current. The electrical pulses are proposed to destabilize transmembrane potential of cells, leading to the formation of so-called ‘nano-pores’ in the cellular membrane. In theory, due to the resulting increased cell membrane permeability, the cell loses its homeostatic properties, resulting in cell death. Recent studies have shown that the therapeutic application of IRE inevitably results in secondary Joule heating and will induce thermal damage. To gain understanding of the temperature development and thermal distribution during IRE, we investigated in Chapter 3 the mechanical effects, changes in temperature gradient and absolute temperature changes, measured with three different optical techniques (high speed-, color Schlieren- and infrared imaging).

Some recommendations of chapter 2 were used to develop an appropriate trial protocol to determine the safety and efficacy of irreversible electroporation (IRE) for the ablation of prostate cancer. In Chapter 4, the protocol of the IRE pilot-study was described. In this multicentre study, sixteen patients with prostate cancer who were scheduled for a radical prostatectomy undergo an IRE procedure, approximately 30 days prior to the radical prostatectomy. Data as adverse events, side effects, functional outcomes, pain and quality of life were collected and patients were controlled at one, two and four weeks post-IRE. Prior to the IRE procedure and the radical prostatectomy, all patients underwent a multiparametric MRI and a contrast-
enhanced ultrasound of the prostate. The efficacy of ablation was determined by whole mount histopathological examinations, which were correlated with the imaging of the ablation zone. Outcomes of this study are comprehensively interpreted and reported in further chapters.

In the evaluation of the treatment effects after focal therapy, imaging plays an important role. In Chapter 5, we studied different imaging modalities for the visualization of IRE effects. The aim was to determine the most feasible imaging modality to visualize IRE ablation zone accurately and to compare the volumetric IRE ablation zone on imaging with ablation volumes on histopathology.

These histopathological results were used in Chapter 6 to assess the IRE ablations and to compare the cross-sectional ablated areas, derived from the histopathology slides, with the areas within the electrode configuration of the IRE planning.

The main purpose of focal therapy is to reduce treatments’ morbidity while ensuring at least equivalent oncologic outcomes when compared with conventional therapies as radical prostatectomy or radiation. Therefore quality of life and patients’ satisfaction as well as the safety of the treatment is of utmost importance to determine after focal therapy. These items were assessed during the short-term follow-up until the planned radical prostatectomy following IRE. The results are presented in Chapter 7.

After the surgery, all prostatectomy specimens were examined to determine the histological changes associated with IRE treatment. Macroscopy and microscopy results were evaluated of the ablated and non-ablated area and presented in Chapter 8. Furthermore, detailed analysis was performed of the adjacent vital structures of the prostate.

This thesis represents to a great extent a phase I-II trial of IRE in prostate cancer patients. All available focal therapies are still considered experimental in the European and American urological guidelines. Key reason is that the focal therapy trials are at the early stage of clinical development, frequently with retrospectively analyzed data and without long-term oncologic evaluation. Chapter 9 gives an overview of the recently published focal therapy trials and advocates what is needed to make focal therapy and accepted segment of standard therapy.

Chapter 10 provides a summary and the conclusions of this thesis and it considers future perspectives.
REFERENCES


Chapter 2

Focal Therapy in Prostate Cancer: International Multidisciplinary Consensus on Trial Design

W van den Bos, BG Muller, H Ahmed, CH Bangma, E Barret, S Crouzet, SE Eggener, IS Gill, S Joniau, G Kovacs, S Pahernik, JJMCH de la Rosette, O Rouvière, G Salomon, JF Ward, PT Scardino

Published in European Urology 2014;65:1078-1083
ABSTRACT

Background Focal therapy has been introduced for the treatment of localised prostate cancer. To provide the necessary data for a consistent assessment, all focal therapy trials should be performed according to uniform, systematic pre- and post-treatment evaluation with well-defined endpoints and strict inclusion and exclusion-criteria.

Objective To obtain consensus on trial design for focal therapy in prostate cancer.

Design, setting, and participants A four-staged consensus project based on a modified Delphi process was conducted in which 48 experts in focal therapy of prostate cancer participated. According to this formal consensus-building method, participants were asked to fill out an iterative sequence of questionnaires to collect data on trial design. Subsequently, a consensus meeting was held, in which 13 panellists discussed acquired data, clarified the results and defined the conclusions.

Outcome measurements A multidisciplinary board from oncological centers worldwide reached consensus on patient selection, pretreatment assessment and evaluation of outcome and follow-up.

Results and limitations Inclusion criteria for candidates in focal therapy trials are patients with PSA < 15 ng/mL, clinical stage T1c-T2a, Gleason score 3 + 3 or 3 + 4, life expectancy of > 10 years and any prostate volume. The optimal biopsy strategy includes TRUS-guided biopsies, to be taken between 6 to 12 months post-treatment. The primary objective should be focal ablation of clinically significant disease with negative biopsies at 12 months post-treatment as the primary endpoint.

Conclusions This consensus report provides a standard for designing a feasible focal therapy trial.

Patient summary A variety of ablative technologies have been introduced and applied in a focal manner for the treatment of prostate cancer. In this consensus report, an international panel of experts in the field of prostate cancer determined pre- and post-treatment work-up for focal therapy research.

Take Home Message This consensus project may be helpful in focal therapy trial design of prostate cancer, it contains agreement on patient selection, pretreatment assessment and evaluation of outcome and follow-up, leading to improved uniform design of clinical focal therapy trials.
INTRODUCTION

Stage migration in localized prostate cancer (PCa) has led to a more significant potential role for focal therapy as a less invasive procedure in the management of the disease [1]. This increased detection rate is partially due to intensified PSA testing, improved imaging technologies and increased public awareness [2] and [3]. A variety of ablative energies have been introduced and applied in a focal manner for the treatment of PCa. These include cryotherapy, high-intensity focused ultrasound (HIFU), laser ablation therapy, radiofrequency ablation, irreversible electroporation (IRE), and photodynamic therapy (PDT). The first two modalities are mentioned as true and experimental therapeutic options in patients with clinically localised prostate cancer by the European Urology Association Guidelines. Although focal therapy is not yet the standard for organ-confined prostate cancer, it is the therapeutic approach with the most important future potential [4]. Currently different approaches to focal therapy have emerged, with each using a variety of patient selection criteria, endpoints and protocols for evaluation and follow-up. It is clear that intra- and inter-technology variability’s are numerous [5] and [6]. There are conflicting recommendations and lack of consensus on the design of focal therapy trials, making it difficult to compare outcomes. Together with debate about what is meant by focal therapy and a divergent view of what is deemed a successful outcome, it is difficult to assess the current state of the field and to determine a clear path forward [7], [8] and [9]. Focal therapy needs mature oncological follow-up data and therefore standardisation, clear definitions of eligibility criteria, and endpoints [10]. To provide the necessary basis for assessing scientific progress, focal therapy trials should be performed according to a uniform systematic pre- and post-treatment evaluation, well-defined endpoints, and strict inclusion and exclusion criteria. The objective of the present study was to develop consensus on focal therapy trial design in prostate cancer. This is, to our knowledge, the first report from an experts’ consensus project to address the issue of focal therapy trial design in prostate cancer.

MATERIALS AND METHODS

Consensus process
This four-staged consensus project is derived from the “Delphi method”. Developed in the 1950s as an instrument to predict the future in political-military, technological and economic topics [11]. Nowadays, the Delphi approach is widely applied for
evaluation of expert opinion on health and medical subjects [12] and [13]. It is a method for consensus-building by using a sequence of questionnaires to collect data from selected subjects [14]. The method generally involves multiple rounds of questionnaires, in which answers are given anonymously. The results of the online questionnaire (using http://www.surveymonkey.com; accessed April 28, 2013), including participants’ comments, were collected and reported back to the group. This feedback process allowed and encouraged the participants to reassess their initial judgments. Consequently, each participant was asked to complete the questionnaire again. For this study, the process was iterated three times to obtain a convergence of opinion on the subject.

**Expert representation**

A systematic literature search of Pubmed database was conducted through 10 April 2013 with pre-specified English language and human-studies restrictions. The search strategy was as follows: “PCA” OR “prostatic neoplasms” OR “prostate cancer” OR “prostate carcinoma” AND “focal treatment” OR “focal therapy” OR “tissue-preservation/preservation” OR “subtotal” OR “cryosurgery” OR “cryotherapy” OR “cryoablation” OR “high-intensity focused ultrasound ablation” OR “HIFU” OR “photodynamic therapy” OR “PDT” OR “laser therapy” OR “brachytherapy” OR “irreversible electroporation” OR “IRE”. In addition, registered trials were retrieved from trials registries (ClinicalTrials.gov and the International Standard Randomised Controlled Trial Number). The results of this search were used to construct the questionnaires. After reviewing the literature and trials, a group of 48 experts in the field of focal therapy in prostate cancer from Europe, United States and Asia were invited to participate in this consensus project. Selection was based on publication record, academic interest, and current practice in their respective fields. This group has an overall experience in performing over approximately 1500 prostate cancer focal therapy procedures in total per year. All experts were requested to submit their protocols of future, currently conducting, or completed focal therapy trials. In this consensus study, members of the following societies took part: the European Organization for Research and Treatment of Cancer-Genito-Urinary Group (EORTC-GU), the American Brachytherapy Society (ABS), the European Society of Therapeutic Radiology and Oncology (ESTRO), the European Association of Urology Section of Urotechnology (ESUT), the European Association of Urology Section of Urological Imaging (ESUI), the Society of Urological Oncology (SUO) and the Endourological Society (ES). The experience of the experts per focal therapy is shown in Table 1. The response rate of the questionnaires was 88%, 85% and 96% in Rounds one, two and three, respectively.
Table 1. Experience of experts per focal therapy

<table>
<thead>
<tr>
<th>Therapy</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryotherapy</td>
<td>64%</td>
</tr>
<tr>
<td>High-intensity Focused Ultrasound</td>
<td>60%</td>
</tr>
<tr>
<td>Brachytherapy</td>
<td>43%</td>
</tr>
<tr>
<td>Laser Therapy</td>
<td>31%</td>
</tr>
<tr>
<td>Photodynamic Therapy</td>
<td>31%</td>
</tr>
<tr>
<td>Radiofrequency Ablation</td>
<td>12%</td>
</tr>
<tr>
<td>Irreversible Electroporation</td>
<td>17%</td>
</tr>
<tr>
<td>Other</td>
<td>5%</td>
</tr>
</tbody>
</table>

**Consensus meeting design**

As the final round of the Delphi process, a consensus meeting was planned on 29th of May 2013, at the beginning of the 6th International Workshop on Focal Therapy and Imaging in Prostate and Kidney Cancer (Amsterdam, the Netherlands: http://www.focaltherapy.org). Participants in the survey, that were attending this meeting, were invited to join the consensus meeting. The meeting was attended by 13 panelists representing the specialties of urology (12), surgery and interventional science (1), radiation therapy (1), radiology (1) and surgery (1). It was chaired by Dr. Peter Scardino (New York, USA).

During this final consensus meeting, all results of the Delphi study were presented and discussed. The panelists were given the opportunity to deliberate on the outcomes on the basis of the results of the literature search. There was the possibility to give feedback on the group’s responses and to address on inconclusive results due to clinical disagreement or eventual misinterpretation.

**Level of Evidence**

This consensus project is based on expert opinions acquired by the Delphi method. The level of evidence is 5 [15].

**RESULTS**

Consensus was reached on a significant number of topics in relation to candidate selection for a focal therapy trial. The appendix summarizes the full questionnaire including post-questionnaires results for all topics. It is essential to agree upon the definition of focal therapy. The panel reached agreement on the following ablative templates, targeted ablation, hemi-ablation and zonal ablation of the prostate (Figure 1).
The panel agreed the pathologist should specify the amount of tumour involvement per biopsy core. The percentage of positive samples should be taken into account in selecting candidates. Tumours with Gleason score 3 + 3 are eligible, containing at least one core with presence of substantial amount of cancer. Consequently, focal therapy should preferably not be offered to patients with clinically insignificant disease who may not benefit from active treatment and in whom focal therapy could be considered as overtreatment. Carcinomas with a Gleason score 3 + 4 and locally confined to the prostate, may be considered as candidates for focal therapy. For patient selection one may consider to include nomograms to minimize inclusion of patients with nodal disease, especially in patients with intermediate disease [16]. The age of the candidate and the PSA density should not be classified as inclusion criteria. Furthermore, there is no limit to the volume of the prostate. In case of HIFU treatment, it is recommended to treat prostates up to 40 cc only [17].

The panelists agreed that 5-alpha-reductase inhibitors needs not be stopped before entering a focal therapy trial. Lastly, it is not mandatory to have MRI-visible lesions concordant with biopsy before including a patient into a trial.

The panel recommended the in- and exclusion criteria for a focal therapy trial as shown in Table 2 to be the minimal required. It is important to be aware that this consensus project on focal therapy trial design should be adjusted in the case of focal brachytherapy. Brachytherapy is a technique that requires a different patient selection, described by Langley et al. [18].

Agreement was reached not to include the following exclusion criteria: renal insufficiency, history of acute/chronic prostatitis, significant erectile dysfunction, and incontinence. Exclusion of individual patients should be based on good clinical judgment, being mindful of all co-morbidities and performance status (e.g., heart disease and concurrent cancers).
Table 2. Inclusion and exclusion criteria for focal therapy trials

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PSA PSA &lt; 15 ng/mL</td>
<td>Previous treatment:</td>
</tr>
<tr>
<td>PSA &gt; 15 ng/mL should be counselled with caution</td>
<td>Previous treatment of the primary cancer within the prostate</td>
</tr>
<tr>
<td>Clinical Stage T1c - T2a</td>
<td>Previous hormone treatment for PCa within 6 months before trial</td>
</tr>
<tr>
<td>Pathology Gleason score 3 + 3</td>
<td>Previous radiation to pelvis</td>
</tr>
<tr>
<td>Gleason score 3 + 4</td>
<td>Active urinary tract infection</td>
</tr>
<tr>
<td>Life expectancy &gt; 10 years [35]</td>
<td>Radiologic imaging:</td>
</tr>
<tr>
<td>Prostate volume Any. Except in case of HIFU: &lt; 40 cc</td>
<td>PI-RADS score &lt; 3; Clinically significant cancer is equivocal ([26])</td>
</tr>
<tr>
<td></td>
<td>Extracapsular extension or seminal vesicle invasion</td>
</tr>
<tr>
<td></td>
<td>Lymph node or bone metastasis</td>
</tr>
</tbody>
</table>

PSA: Prostate-specific antigen. HIFU: High-intensity Focused Ultrasound. PCa: Prostate cancer. PI-RADS: Prostate Imaging Reporting and Data System.

These criteria are the minimal requirements in including and excluding candidates in focal therapy trials.

**Endpoints**

The primary endpoint of focal therapy trials should be focal ablation of clinically significant disease (defined as prostates with a dominant tumour > 0.5 cc) with negative biopsies evaluated at 12 months post-treatment as the primary endpoint. The clinical validity of MRI to analyse the presence of residual or recurrent cancer compared to histopathological findings should be a secondary endpoint of the trial. Alterations on MR-imaging itself are not a sufficient endpoint. In case of focal brachytherapy, a more prolonged schedule of follow-up should be accomplished [18].

The panel agreed that PSA levels should be monitored but should not be included as one of the endpoints. Since the utility of PSA kinetics in tissue preservation treatments is yet to be determined [6] and [19].

**Pretreatment assessment**

The panel agreed that a prostate-specific antigen (PSA) measurement is essential, but testing of any PSA derivatives (e.g., PSA–velocity, -doubling time, -density) is not. The International Index of Erectile Function (IIEF) -score and International Prostate Symptom...
Score (IPSS) are preferable questionnaires to assess patients’ functional status. The experts were in agreement that quality of life should preferably be quantified by the Expanded Prostate Cancer Index Composite (EPIC). In case of incontinence, the use of pads should be reported. Also performing uroflowmetry is not essential but may provide additional data on outlet obstruction and post-voiding residual, which can (temporarily) worsen following focal therapy.

Biopsies and Imaging

Literature states that performing systematic TRUS-guided biopsy alone is insufficient for the purpose of selecting candidates and determining the exact location of the disease for focal therapy [20], [21], [22], [23] [24] and [25]. Subsequently, it was agreed that MRI/TRUS fusion-guided targeted biopsies should be performed in addition to systematic TRUS-guided biopsies. Moore et al. have made a notable effort to develop standards for reporting of MRI-targeted biopsy studies. Which are considered useful concerning in the pretreatment assessment in focal therapy [26]. If MRI cannot be performed prior to the biopsies, it is recommended to allow an interval of at least four to eight weeks between biopsies and MRI-scan to reduce image misinterpretation from biopsy-induced artifacts.

It was recommended by the panel to perform an MRI-scan with the best MRI characteristics available, following the ESUR guidelines and consensus recommendations as much as possible [27] [28]. Awaiting the ultimate tool for detecting prostate cancer, multi-parametric MRI (T2WI combined with at least two functional MRI techniques) with its detection rate up to 97% is an accurate tool for identifying and quantifying intracapsular clinically significant tumour foci [29]. Because of the high negative predictive value (up to 95%) for clinically significant tumours, multiparametric-MRI (mp-MRI) is a useful tool to detect aggressive tumours (e.g., high-volume Gleason 4+3 or higher), which should be excluded in trials (Table 2). Furthermore, mp-MRI can be used to distinguish which zones of the prostate do not require therapy [30] and [31].

Preplanning and preparation

It was agreed that the following MRI sequences are useful in the preplanning on focal therapy: T2MRI for assessing the anatomy, diffusion-weighted imaging (DWI) for specifying lesion characteristics, and dynamic contrast enhanced MRI (DCE-MRI) for increased cancer detection. Before commencing focal therapy, antibiotic prophylaxis should be administered combined with the placement of an indwelling urethral catheter. It was agreed that MR-spectroscopy, real-time or shear wave
elastography, contrast-enhanced ultrasound and HistoScanning might be useful for preplanning, because it may provide information about location, aggressiveness and extend of the tumour [32] [33] and [34].

Evaluation of outcome and follow-up

**Oncological outcomes**

There was consensus that it is essential to measure PSA and to perform periodic biopsies during follow-up. Post-treatment PSA follow-up should be performed at three month intervals during the first year, biannually in the second year, and annually in the third year. Thereafter, the frequency of checking PSA is at the discretion of the investigators. The panel was in agreement that the optimal biopsy strategy includes TRUS-guided systematic whole-prostate biopsies and additionally targeted biopsies, to be taken between 6 to 12 months post-treatment. This interval allows resolution of inflammatory effects and formation of scar tissue. In case of clinical suspicion, it is advisable to perform biopsies in case of clinical suspicion only. Uro-oncological experience is an agreed on requirement for any pathologist analysing prostate biopsies. All biopsy results should be reported in detail, including the number and location of cores taken and the number of positive cores, as well as the amount per biopsy of the involved cancer (in mm). In case of in- or out-of-field recurrent or residual disease, one-time retreatment within the trial is acceptable. MRI, preferably mp-MRI, should be performed before taking the biopsies (or at least 6-8 weeks afterwards) and should be assessed by trained uro-oncological radiologists. However, this should be predetermined and described in the protocol.

**Functional outcomes**

It was agreed to measure functional status, quality of life and adverse events as part of the follow-up. Although, no consensus could be made about administering anxiety-scores, it was mentioned to be interesting to measure anxiety in follow-up of focal treatment.

**Treatment failure**

In-field failure is defined as: 1) Cancer of higher Gleason grade or 2) persistent cancer of similar or lower grade after repeat focal therapy to the same area or 3) the need for additional PCa treatment other than focal therapy because of objective findings elsewhere in the gland (e.g., high-grade cancer). Low-grade, low volume tumour foci (< 3mm Gleason 3 + 3) found out-of-field are not designated as failure. Moreover, out-of-field disease with tumour characteristics as described in Table 2 (in inclusion criteria)
was designated as selection failure. These latter groups are not to be excluded from further evaluation. The choice of retreatment after failed focal therapy depends on the stage and grade of the recurrent tumour.

The panel was in agreement that an accurate definition of biochemical failure cannot be made because of insufficient data.

Both phase II and phase III trials are currently underway in the focal therapy field. Favored duration of future prospective phase II (single-arm) studies is 18-36 months and prospective phase III (randomized) comparative studies is 3 to 5 years. In randomized clinical trials, stratification should be based on PSA, stage and grade of the tumour, and the amount of cancer in systematic and targeted needle biopsies.

**DISCUSSION**

All recommendations were created by the Delphi method, which is based on experts opinions and therefore contains this paper only level 5 evidence [15]. Since the contributors were selected for their expertise in focal therapy, they were possibly biased by their enthusiasm. Furthermore, the low number of the participating panelists out of the large group of survey contributors is a potential limitation. However, the relatively high level of consensus (Appendix) indicates that there was a pre-existing notion on the design of focal therapy trials. This project evaluated these common grounds and extracted recommendations for future research. No consensus was determined on certain topics such as schedule standardization of functional status, quality of life scores and adverse event reporting.

**CONCLUSIONS**

In conclusion, this paper describes a multistage Delphi process agreement on patient selection, pretreatment assessment, evaluation of outcome and follow-up. This consensus report provides a standard for focal therapy trial design, which is considered important since clinical trials have been conducted with such different design characteristics that the outcomes are regarded scarcely comparable. The recommendations were made in consultation with a multidisciplinary board from oncolgical centers worldwide.

Appendix with the results of the questionnaires are presented in the Supplements.
REFERENCES


Chapter 3

Thermal energy during irreversible electroporation and the influence of different ablation parameters

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Published in Journal of Vascular and Interventional Radiology 2015 Dec. 17
ABSTRACT

Purpose Irreversible electroporation (IRE) uses high-voltage electric fields to achieve cell death. Although the mechanism of IRE is mainly designated as non-thermal, development of secondary Joule heating is inevitable. The study purpose was to gain understanding of the temperature development and distribution during IRE.

Materials and Methods IRE was performed in a transparent polyacrylamide gel resembling soft-tissue. Mechanical effects, changes in temperature gradient and absolute temperature changes were measured using three different optical techniques (high speed-, color Schlieren- and infrared imaging). We investigated the effect on the temperature of variations in voltage, pulse length, active tip length, interelectrode distance, electrode configuration (parallel, convergent and divergent) and sequential pulsing (pulse delivery interrupted by breaks). The total delivered energy was calculated.

Results A temperature gradient, starting at the tips of both electrodes and expanding towards each other, developed immediately with pulse delivery. Temperatures rose with increasing voltage (ΔT 2.5-40.4°C), pulse length (ΔT 5.3-9.8°C), active tip length (ΔT 5.9–17.6°C) and interelectrode distance (ΔT 7.6–21.5°C), in accordance with higher energy delivery. Non-parallel electrode placement resulted in heterogeneous temperature distribution with the peak temperature focused in the area with the shortest interelectrode distance. Sequential pulse delivery significantly reduced the temperature increase compared to continuous pulsing (ΔT 4.3 vs 11.7°C).

Conclusions Voltage, pulse length, interelectrode distance, active tip length and electrode configuration each have a strong effect on the temperature development and distribution during IRE. Sequential pulsing reduces the extent and volume of thermal distribution and may prove beneficial with respect to procedural safety.
INTRODUCTION

Irreversible electroporation (IRE) is increasingly used for the ablation of malignancies, in particular tumors located near vulnerable structures, such as bile ducts and blood vessels. The technique utilizes high-voltage microsecond electrical pulses, applied through adjustable needle electrodes. The electrical pulses are proposed to destabilize the existing cellular transmembrane potential, leading to the formation of so-called ‘nano-pores’ in the cellular membrane. In theory, due to the resulting increased cell membrane permeability, the cell loses its homeostatic properties, resulting in cell death [1]. Because the IRE mechanism induces cell death by affecting the cellular membrane, cells are killed in a targeted region, without damaging the collagen and other interstitial tissue constituents. Critical structures like major vasculature and ductal systems may therefore be preserved [2,3]. The sparing of critical structures is the primary characteristic that distinguishes IRE from other local therapies, offering a therapeutic option for targeting tissues that are contra-indicated for surgical resection, thermal ablation, or radiation therapy [4]. In the light of this important benefit, IRE has shown promising results in the ablation of centrally located hepatic, locally advanced pancreatic and prostate tumors [5–7].

The formation of nanoscale defects occurs independently of thermally-induced processes, and the non-thermal mechanism was demonstrated in a large soft tissue sarcoma and during an intracranial procedure [8,9]. Nonetheless, recent studies have shown that the therapeutic application of IRE will result in secondary Joule heating that can induce thermal damage [2,3,10,11]. Given the fact that the underlying rationale for the clinical application paradigms of IRE are in large part based on the assumption of the non-thermal mechanism of cell death, characterization and quantification of the thermal effects of IRE is necessary to ensure safe but effective ablations [2].

We hypothesize that thermal distribution during IRE ablations varies in respect to the ablation settings depending on the amount of the delivered Joules. The heating effects will initially be observed around the needles and will subsequently merge after substantial energy delivery. Additionally, it was hypothesized that sequential pulsing results in a smaller increase of temperature than consecutive pulse delivery, because of the intermittent cooling periods without Joule delivery. It was furthermore hypothesized that electrolysis of water might occur during IRE pulsing in water-based gel, because by using direct current through an ionic substance, an interchange of ions takes place causing this effect. The primary goals of this study are the visualization of physical effects of IRE pulses, quantification of the development and distribution of thermal energy during IRE using optical approaches and to determine the influence...
of different ablation parameters on the thermal outcome. The secondary goals of this study are the evaluation of the effect of sequential (pulse delivery interrupted by breaks) versus consecutive pulse delivery and the evaluation of non-parallel electrode placement on the thermal effect during IRE. The further identification of the thermal component of IRE is vital for the improved safety of IRE in interventional oncology.

MATERIALS AND METHODS

Tissue mimicking gel phantom
We used a transparent polyacrylamide gel of which the characteristics mimic soft biological tissue with respect to mechanical properties, and electrical and thermal conduction [12]. For 100 ml gel we used 60 ml saline (NaCl 0.9%), 50 mg ammonium persulfate, 40 ml 30% acrylamide/bis solution and 80 µl tetramethylethyleendiamine. The gels were casted by pouring the liquid material into a fixed mold. Dimensions of the gel were 10 cm width, 8 cm height, and 1.5 cm thickness, which allowed for electrode placement similar to in-vivo settings.

IRE-procedure
The IRE-procedure was performed using the Nanoknife® IRE console (AngioDynamics, Latham, New York). For the standard ablation setting, two monopolar 19-gauge needle electrodes were placed in the gel, exactly parallel using a grid and 5 +/- 1 mm from the gel surface (Figure 1A- B). The proximal aspect of the active tip was constantly ~4 cm from the top surface. The default ablation settings were 15 mm interelectrode distance, 15 mm active tip length (ATL), delivering 1×90 pulses with a pulse length of 90 µsec, 90 pulses/minute and pulse intensity of 1000 V/cm. The influence of the following ablation parameters on the temperature was objectified: Voltage (ranging from 500 to 2500 V; interelectrode distance 10mm), pulse length (50, 70 and 90 µsec), interelectrode distance (ranging from 5 to 25 mm for 1000 V/cm), ATL (ranging from 5 to 25 mm) and electrode orientation (parallel, divergent and convergent). Also, continuous delivery of 120 pulses (2 × 60 with an 18 seconds inevitable pause required for the generator recharge) was compared to sequential pulse delivery (4×30 pulses and 2x60 pulses interrupted by breaks of 30, 60 and 90 seconds), as was the effect of converging and diverging electrodes under a 45° angle.
Thermal energy during irreversible electroporation

Figure 1. (A) Close-up image showing thermal camera and inserted electrodes in gel. (B) Schematic image of the electrodes in the gel with standard settings (interelectrode distance of 15 mm and active tip length of 15 mm). (C) Analysis of temperature recordings with three regions of interest.

Physical effects of IRE
To visualize physical phenomena during individual pulses, 10 IRE pulses were registered using a high-speed camera (Photron Fastcam MC2, San Diego, CA, USA) mounted in front of the gel showing a 20 mm diameter close-up image of the electrodes capturing images at a frequency of 50 to 8000 frames per second (time resolution from 20 ms to 125 μs) [12].

Temperature gradient measurements
The initial temperature effects during the first pulses (which also reflect the position of the highest currents along the needle surface) were registered using the color Schlieren imaging technique [13,14]. This technique allows visualization of small changes in optical density, induced by temperature gradients or local stresses. The degree of deflection of the parallel light rays, caused by the temperature gradient, is color coded by a spatial filter (rainbow filter), resulting in a qualitative pseudo ‘thermal’ image recorded by the high-speed camera (Figure 2) at 250 f/s (4 ms/frame).

Absolute temperatures measurements
The effect of variation in ablation settings on the absolute temperature and distribution at the gel surface was investigated using a Xenics Gobi-384 thermal camera (Xenics, Leuven, Belgium). This infrared camera uses long wavelengths (8-12 μm range) to record thermal changes of a minimum of 0.05 °C at a resolution of 384 × 288 pixels (25-μm pitch). The camera is calibrated at a range of -20°C to 120°C [11].
Analysis and statistics

Reproducibility, by means of average temperature difference and standard deviation over multiple measurements (5x), was tested using default settings. The results of the experiments proved to be consistent within 1 °C, which was used as the standard error within the experiments. After the validation of the investigational setup, the subsequent experiments were performed once to observe general trends between parameters. For each experiment, three ROI’s were selected of ~ 100 pixels per ROI to follow temperature over time. The ROI with the highest mean temperature at the most intense portion of the image was used to calculate the temperatures. Each individual measurement point in time is acquired with a minimal and maximal temperature per ROI (Figure 1C). The difference between this minimal and maximal temperature was used to calculate the percentile error for each individual data point. Herewith, the average error for all data points in time was calculated. Because the baseline temperature of the gel varied (ranging from 12 to 16 °C), the relative phantom surface temperature ($\Delta T$) was determined between the start ($T_0$) and maximal measured temperature of each ablation. The change in delivered current ($\Delta A$) as determined by the IRE-console was noted. The resultant total of delivered energy $E$ in Joule was calculated using the following formula:

$$E = V \times I \times N_p \times t_d$$  \hspace{1cm} (1)
Thermal energy during irreversible electroporation

Where $V$ is the applied voltage, $I$ is the used current in amps, $N_p$ is the number of pulses and $t_d$ is the pulse duration time in seconds.

RESULTS

**Physicals effect of IRE**

During each pulse a light flash was visible at the negative electrode and small gas bubbles were formed predominantly along the negative electrode (Figure 3A). Vapor formation, resulting in cavities in the gel focused in the converged tip of the negative electrode. The cavity enlarged in volume with each pulse, related to the amount of delivered energy per pulse, with a maximum of 2.4 mm (Figure 3B).

![Figure 3. High-speed camera images showing (A) Start of electric current with light flash at the negative needle electrode (125 f/s) (B) Vapor formation during the first three consecutive pulses at the negative electrode (1000 f/s). The background shows the light source.](image)
Temperature gradient measurements

The temperature gradient distribution is shown in figure 4A-D during 10 pulses. The initial gradient reflects the area of the highest current density at the electrode tips (4B), expanding alongside the entire active length. The temperature field builds up with each pulse (4C) and decreases after completion of the last pulse (4D). No discrepancy was observed between the negative and the positive electrode.

Figure 4. High-speed color Schlieren images showing the temperature gradient distribution during (A-C) an IRE pulse train of 10 pulses and (D) during the subsequent relaxation using default settings (15 mm interelectrode distance, 15 mm ATL, delivering 1×90 pulses with a pulse length of 90 µsec, 90 pulses/minute and pulse intensity of 1000 V/cm).

Absolute temperature measurements

Figure 5 shows the thermal images of the maximum temperatures measured at the gel surface for each voltage. A regular increase in $\Delta T$ was observed with higher voltages. After each IRE-ablation, a rise in current ($\Delta I$) was detected resembling the current increase reported during clinical IRE procedures. Similar to the temperature effect, the rise in amperage was positively correlated to the total amount of energy delivered in Joule. A similar effect was seen for pulse length (Figure 6), ATL (Figure 7), and interelectrode distance (Figure 8). The resulting graphs and tables showing the regular temperature increase followed by a decrease (cooling time), when the pulse delivery was completed, are included in the figures and marked with an asterisk.
Figure 5. Temperature (T) and current development (A) over time for various voltages and resultant dissipated energy. * Represents the end of pulse delivery.
Figure 6. Temperature (T) and current development (A) over time for various pulse lengths and resultant dissipated energy. * Represents the end of pulse delivery.
Figure 7. Temperature distribution and temperature (T) and current development (A) over time for various active tip lengths and resultant total dissipated energy. * Represents the end of pulse delivery.
Figure 8. Temperature ($T$) and current development ($A$) over time for various interelectrode distance with 1000 V/cm and resultant total dissipated energy. * Represents the end of pulse delivery.
In Figure 9, all temperature measurements are plotted against the total delivered energy for a constant interelectrode distance (15 mm). In first order approximation, the energy \( E \) is dissipated in a constant volume \( V \) around the electrode resulting in a temperature increase with the linear relation: \( \Delta T = \frac{E}{V \times 4.2 \text{ J}} \) (in which 4.2 J represents the amount of energy required to heat up 1 cm\(^3\) of water with 1 °C). The volume \( V = 4.7 \text{ cm}^3 \) is derived from the linear fitting. This volume is in good accordance assuming a cylindrical volume heated around each electrode with a diameter of 13 mm and a length of 15 mm (2 x 2.3 cm\(^3\)).

Continuous pulsing with 120 pulses resulted in a rise of almost 12 °C. However, when pulses were delivered sequentially, the temperature decreased during each break, resulting in a significant reduction of the \( \Delta T \). Furthermore, by extending the pause between the pulse series, the resultant \( \Delta T \) was lower. The table shows the amount of dissipated energy, which is constant for each ablation (Figure 10).
With converging as well as with diverging electrodes in a 45° angle, the temperature focused in the area where the electrodes were closest to each other (5 mm). The total temperature increase in this focal point was higher than the total temperature increase for exact parallel electrode placement (Figure 11).
The thermal camera measures temperatures at the gel surface at 5 mm distance from the electrodes. Therefore, the actual absolute temperature at the electrode surface is higher than displayed. Extrapolation of the temperatures at 3, 5, 10, 15 and 20 mm from the electrodes results in an exponential curve, which illustrates that the temperature closer to the electrodes is much higher, and quickly decreases further away from the electrodes (Figure 12).

**Figure 11.** The temperature distribution during (A) parallel, (B) divergent, and (C) convergent electrode placement (5 mm interelectrode distance).

**Figure 12.** Exponential curve showing the temperatures at different distance to the electrodes.
DISCUSSION

Recently, the nomenclature regarding IRE as a non-thermal ablation technique was extensively refuted [3,11,15]. While cell-scale IRE effects are well described to provide a non-thermal method for cell death [16–18], there is substantial evidence that pulse protocols of IRE in the clinical setting induce thermal damage within the ablation zone [2, 7, 8, 12]. Thermal damage is a function of temperature and exposure duration, and care must be taken to ensure that the cumulative effects do not induce damage to the heat-susceptible structures in the vicinity of the ablated region that could lead to complications [3,4]. To the best of our knowledge the exact distribution of thermal energy around the electrodes during ablation has not been visualized before. Our data confirm that IRE causes a substantial temperature increase (59.7 °Celsius).

Gas formation
The development of gas bubbles from the start of the first pulse accompanied by the flash at the tip of the negative electrode, as visualized by the high-speed camera, indicates very high local temperatures. This results in explosive vaporization of water at the electrode tip followed by a rebound implosion due to subsequent condensation, temporarily creating a small cavity in the gel. Subsequently, due to the electric current passing through the saline-based gel, it is likely that an electrolytic process (decomposition of water \([\text{H}_2\text{O}]\) into oxygen \([\text{O}_2]\) and hydrogen gas \([\text{H}_2]\)) occurs. Both effects probably contribute to the formation of the bubbles.

Distribution and geometry of thermal effects
Although the first order approximation of the dissipated energy reliably predicts the temperature increase, an exponential temperature gradient exists between the electrode (i.e. the thermal source) and the surrounding environment [19], as shown in figure 12. Extrapolation to the actual electrode surface suggests a substantially higher local temperature. Furthermore, when electrodes are placed asymmetrically, the thermal effect focuses in the area with the shortest interelectrode distance. Given the time-temperature relationship for heat-induced killing following the Arrhenius equation [20], a temperature increase up to 10 °C for several minutes seems acceptable. For these reasons we recommend a minimum electrode distance of 5mm (Figure 12) from critical structures and even greater near the focus of the active tips for angulated pairs. Furthermore, since increasing interelectrode distance (at constant V/cm) generates higher local temperatures, we advise to keep the interelectrode distance rather small (i.e. 10-15 mm), to better control the temperature when IRE is performed near critical structures.
**Pulse sequences**
Sequential pulsing with pulse trains of 20 or 30 pulses significantly reduced the temperature increase, depending on the duration of the breaks. Aside from the fact that lower temperatures improve procedural safety, Appelbaum et al. recently showed that multiple shorter cycles of energy application using a four-probe array created larger ablation zones, at the cost of increased treatment duration [21]. The authors hypothesized that the increase in electrical conductivity induced by an IRE pulse persists after the initial pulse. The shifts of cellular contents such as solutes, caused by the opening of IRE-induced pores in the cellular membrane, occur in the order of minutes rather than seconds. So, besides the number of pulses at a given voltage alone, timing might also influence ablation zone volume. To this extent, IRE may behave similarly to cryoablation where freeze-thaw cycles have been shown to increase the zone of cell death [22]. In the clinical setting, 3-6 electrodes are generally used for ablation, resulting in 3-11 electrode pairs. If trains of e.g. 20 pulses are applied to each of the electrode pairs in a repetitive, cyclical fashion, this will automatically result in a long pause between pulse-sets for individual electrode pairs, so no additional pause would need to be incorporated, so procedure duration can be controlled.

**Conductivity**
Interestingly, similar to ablation of in situ human tissue, we too noted a steady rise in amperage during each pulse ablation cycle. In the used acellular gel, this is clearly not ascribed to increased cell membrane permeability. A possible explanation for the increase in conductivity (and decrease in resistance) of the gel during electroporation is the rise in temperature of the gel, implying a conductivity increase with increasing temperature [23]. If this assumption is correct, one could argue that the decrease in tissue resistance during clinical IRE may not (solely) be attributed to increased cell membrane permeability, but may be caused by the increased tissue temperature. Measuring changes in electrical properties of cells has been proposed for determining the effectiveness of electroporation protocols in individual cells and in cell cultures [24–26]. Furthermore, Dunki-Jacobs et al [10] proposed that the decrease in impedance should be used during IRE, and suggested a required current increase of approximately 12-15 amperes for successful ablation, with repetition of the protocol in case of a lower increase. But our study proved that caution should be taken when repeating the electroporation protocol to achieve the desired current rise, since the cumulated energy may cause thermal damage.
Limitations

This study has several limitations. Because the electrical and thermal properties of the gel may differ from normal tissue – e.g. the gel was colder and not perfused – the temperature curves cannot be directly translated into the clinical setting. In-vivo ablations will be influenced by tissue-specific thermal and electrical conductivity resulting in altered temperature distribution. The results of this study should therefore be interpreted as describing important trends only. Analysis of the peaks of the temperature curves occasionally showed an inaccurate registration of the start of the ablation since the maximum temperatures were not always synchronously displayed. Despite these limitations, the obtained temperature and current changes seem to be comparable with in-vivo measurements [10,11]. Although we used a grid to place the electrodes exactly parallel with the desired interelectrode distance aiming at a precise 5 mm distance to the gel wall, a small displacement of ± 1.0 mm may have occurred incidentally, which may have resulted in higher or lower temperatures measured at the gel surface of a few degrees. This might explain the discrepancy we measured for 10 mm interelectrode distance, which resulted in a higher temperature increase compared to 15 mm. Furthermore, because of the short distance of the electrodes to the anterior surface (5 mm), the surface temperature we measured may have been higher than in the clinical setting, since the gel-air boundary possibly limits heat dispersion. Nevertheless, the rise in temperature had a consistent positive correlation with the total amount of Joule energy delivered. An additional limitation is that after the investigational setup had been validated, the experiments were performed once, which limits the possibility of statistical analysis. Furthermore, the true dependence of the temperature development and distribution on interelectrode distance cannot by evidently determined, since the amount of energy was partially defined by the used voltages. Last, it is not known whether the performed ablations would have led to actual and complete cell death of the electroporated tissue in the oncologic setting. To determine this, the experiments should be performed in an in-vivo setting.

The thermal component of IRE surrounding the electrodes, can no longer be ignored and every physician should take this into account when planning and performing clinical IRE. Specifically, in clinical practice even higher voltages are used than in the present study. Researchers are now faced with the challenging task to develop optimal treatment algorithms that are strong enough to create complete cell death, but weak enough to avoid thermal damage in areas where this can have detrimental effects. Nonetheless, it is conceivable that the thermal element of IRE may have a synergistic effect on treatment efficacy. For example, higher temperatures may
increase the size of the actual ablation zone. Also, thermal ablation has been suggested to trigger the release of pro-inflammatory, anti-cancer mediators, which activates the adaptive immune system and elicits an anti-tumor immune response against residual viable cancer cells in the treatment area as well as distal, non-treated cancer cells [27] [28,29]. This concept of a local therapy having a systemic response - the “abscopal effect” - has also been suggested for IRE [30,31]. Maybe a synergistic effect of thermal and electrical cell destruction will induce the greatest anti-tumor effect. The immunologic effect of thermal ablation and IRE is the current focus of several trials.

CONCLUSIONS

In conclusion, during IRE ablations, varying voltage, pulse length, interelectrode distance, active length exposure and electrode configuration all have a significant effect on the temperature development in good correlation with the dissipated energy near the electrodes. To this extent, sequential pulsing reduces the extent and volume of thermal damage and may prove beneficial with respect to procedural safety. In order to ensure complete ablation whilst preventing thermal damage, the oncologic efficacy with the different ablation settings – especially the protocol for sequential pulsing - should be validated in animal and clinical studies.
REFERENCES

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Chapter 4

The safety and efficacy of irreversible electroporation for the ablation of prostate cancer: A prospective human in-vivo study

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Published in British Medical Journal Open 2014;4:e006382
ABSTRACT

**Introduction** Current surgical and ablative treatment options for prostate cancer have a relatively high incidence of side effects, which may diminish the quality of life. The side effects are a consequence of procedure related damage of the blood vessels, bowel, urethra or neurovascular bundle. Ablation with irreversible electroporation (IRE) has shown to be effective in destroying tumour cells and harbours the advantage of sparing surrounding tissue and vital structures. The aim of the study is to evaluate the safety and efficacy and to acquire data on patient experience of minimally invasive, transperineally image-guided IRE for the focal ablation of prostate cancer.

**Methods and analysis** In this multi-centre pilot-study, 16 patients with prostate cancer who are scheduled for a radical prostatectomy will undergo an IRE procedure, approximately 30 days prior to the radical prostatectomy. Data as adverse events, side effects, functional outcomes, pain and quality of life will be collected and patients will be controlled at 1 week and 2 weeks post IRE, 1 day preprostatectomy and post-prostatectomy. Prior to the IRE procedure and the radical prostatectomy, all patients will undergo a multiparametric MRI and contrast-enhanced ultrasound of the prostate. The efficacy of ablation will be determined by whole mount histopathological examination, which will be correlated with the imaging of the ablation zone.

**Ethics and dissemination** The protocol is approved by the ethics committee at the coordinating centre (Academic Medical Center (AMC) Amsterdam) and by the local Institutional Review Board at the participating centres. Data will be presented at international conferences and published in peer-reviewed journals.

**Conclusions** This pilot study will determine the safety and efficacy of IRE in the prostate. It will show the radiological and histopathological effects of IRE ablations and it will provide data to construct an accurate treatment planning tool for IRE in prostate tissue.
INTRODUCTION

Current prostate cancer treatments can cause major side effects including urinary incontinence, erectile dysfunction and bowel urgency. These side effects occur due to damage of the neurovascular bundles, urethra including distal urethral smooth muscle sphincter, puboprostatic ligaments and rectum wall. To avoid damage to these structures, several ablative modalities have been introduced with the aim of effective cancer control without jeopardising functional outcomes. Preclinical studies with irreversible electroporation (IRE), a novel ablative modality, demonstrated an advantage over other focal therapies by effective ablation of tumour tissue, while sparing surrounding tissue and vital structures such as blood vessels, urethra and nerve bundles [1-3]. It has been suggested that the potential of IRE to spare essential structures may help to reduce or even avoid side effects in the focal treatment of prostate cancer. Electroporation is a technique in which electric pulses, travelling between two or more electrodes, increase the permeability of the cellular membranes. This effect can either be reversible or irreversible. Reversible electroporation has been employed in electrochemotherapy, to facilitate the uptake of chemotherapeutic agents into cells. The temporary damage to the cellular membrane allows the chemotherapeutic agent to enter the cell, followed by recovery of the membrane [4,5]. Damage becomes permanent above a certain threshold of electrical pulse length and kV/cm, which causes cell death due to the inability of the cell to maintain homeostasis. Initially, the manifestation of this irreversible component during electroporation was considered an unwanted treatment side effect [6-8].

In recent years, interest in IRE as a tumour ablation modality by inducing irreversible cell damage has risen. IRE has shown to be able to effectively ablate tumour cells in vitro, in animal experiments and more recently in several human safety and efficacy studies for liver, pancreas, pelvis, kidney and lung tumours [9-11]. Two main factors have driven research in IRE as a treatment modality. First, studies in animals and humans have shown that connective tissue structure could be preserved with minor damage to associated blood vessels, neural tissue or other vital structures. Second, IRE lesions show a sharp demarcation between ablated and non-ablated tissue whereas lesions from thermal ablation techniques show a transitional zone containing partially damaged tissue between ablated and healthy tissue, because of partial conduction of heat or cold to the surrounding tissue.

The patients will be assigned into two groups. The first group receives a focal ablation of the prostate (one lobe using 2-3 IRE needles); the second group will have an extended ablation (one or both lobes using >3 IRE needles). This allows us to assess
the endpoints of the study in different template scenarios. The first primary objective is to determine if the IRE ablation procedure is safe as measured by the total number of (1) device related and (2) periprocedural and postprocedural adverse events as measured using the NCI Common Terminology Criteria for Adverse Events (CTCAE). The second primary objective is to determine if complete ablation of the specified targeted ablation zone is achieved as measured by histopathology assessment. The first secondary objective is to determine if procedural side effects associated with current treatments for prostate cancer are avoided as measured by the following validated questionnaires: the five-item version of the international index of erectile function (IIEF-5), international prostate symptom score (IPSS) and if required, time of indwelling catheter. The second secondary objective is to determine quality of life (QoL) and comfort measured by expanded prostate cancer index composite (EPIC) and IPSS QoL score, postprocedural pain management and pain scores using the visual analogue scale (VAS) and length of hospital stay [12]. The final secondary objective is to determine accuracy of ablation zone detection by multiparametric MRI (mpMRI) and contrast-enhanced ultrasound (CEUS).

**METHODS AND ANALYSIS**

**Trial protocol**
Patients with confirmed prostate cancer scheduled for radical prostatectomy (RP) with a life expectancy of at least 10 years will consecutively undergo the IRE procedure approximately 30 days prior to the RP. The time frame is based on animal studies, which report completely dissolved IRE lesions after at least three weeks. Furthermore, it is in line with the Department of Health maximum allowed waiting time criteria as well as the standard time between biopsy and RP. Recruitment will take place in two academic hospitals: Academic Medical Center, Amsterdam, the Netherlands and Sismanoglio hospital, Athens, Greece. The study is approved by the research ethics committees of both hospitals and registered in the clinicaltrials.gov database (NCT01790451).

**Inclusion and exclusion criteria**
Patients who are scheduled for a RP and meet the inclusion criteria (Table 1) will be offered to attend a screening visit. The urologist together with a research nurse will explain the study protocol and the patients’ information brochure will be provided. Patients will be excluded from the study if they meet any of the exclusion criteria...
also listed in Table 1. To rule out cardiac disorders, every patient will undergo electrocardiography. If the patient chooses to participate, the informed consent form has to be signed accompanied by one of the research fellows.

Table 1. Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>Patients with prostate cancer who are indicated to undergo a radical prostatectomy</td>
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<tr>
<td>Life expectancy &gt; 10 years</td>
</tr>
<tr>
<td>Able to visualize prostate gland adequately on transrectal US imaging</td>
</tr>
<tr>
<td>No prostate calcification greater than 5 mm</td>
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<tr>
<td>Ability of subject to stop anticoagulant and anti-platelet therapy for 7 days prior and 7 days post procedure</td>
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<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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</thead>
<tbody>
<tr>
<td>Bleeding disorders</td>
</tr>
<tr>
<td>Active urinary tract infection</td>
</tr>
<tr>
<td>History of bladder neck contracture</td>
</tr>
<tr>
<td>Inflammatory bowel diseases</td>
</tr>
<tr>
<td>Concurrent major debilitating illness</td>
</tr>
<tr>
<td>ICD / Pacemaker /Cardiac History</td>
</tr>
<tr>
<td>Prior or concurrent malignancy</td>
</tr>
<tr>
<td>Biologic therapy for prostate cancer</td>
</tr>
<tr>
<td>Chemotherapy for prostate cancer</td>
</tr>
<tr>
<td>Hormonal therapy for prostate cancer within 3 months of procedure</td>
</tr>
<tr>
<td>Radiotherapy for prostate cancer</td>
</tr>
<tr>
<td>Transurethral prostatectomy or urethral stent</td>
</tr>
<tr>
<td>Prior major rectal surgery</td>
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</table>

**Baseline characteristics**

Physical examination will be performed and validated questionnaires (IPSS, IIEF-5 and EPIC) will be used to report baseline urinary and erectile symptoms as recommended by the International Multidisciplinary Consensus on Trial Design for Focal Therapy [13]. Prostate-cancer related pain is measured on the standardized Visual Analogue Score (VAS), ranging from 0 to 10, with higher scores indicating more severe pain [14]. Postoperative pain management will be determined as well.

**Imaging studies**

*Multiparametric MRI*

mpMRI scans (1.5T including diffusion-weighted imaging and dynamic post contrast MRI) will be performed pre-IRE and post-IRE, but pre-RP using an integrated pelvic phased array endorectal coil to localize the tumour and to be able to evaluate the extent of the ablation zone. The diagnostic accuracy for detecting small low-grade malignant lesions in the prostate is strongly dependent on MRI protocol, MRI quality
and user experience [15,16]. There are many studies in which MRI is compared with RP specimens [17-21]. However, limitations in validating the MRI findings with the ‘gold standard’ whole mount histopathology arose from free hand slicing (deformation) and non-uniform distortion on fixation of the specimens. The orientation of the cutting planes in the prostatectomy specimen can be different from the scanning planes of mpMRI, and it is therefore challenging to assess the true accuracy of MRI. However, Turkbey et al. provided a solution for this issue in 2010 by processing the histopathological specimens exactly according to the MRI by using a customized three-dimensional (3D) mold to slice up the RP specimen from 45 patients. The positive predictive value of 3T mpMRI for the detection of prostate cancer increased to 98%, 98% and 100% in the overall prostate, peripheral zone and central gland, respectively [22].

**Contrast-enhanced ultrasound**

The patients who participate in the AMC Amsterdam will undergo a contrast-enhanced ultrasound (CEUS) pre-IRE and pre-RP to determine the completeness of the ablation zone detection by this imaging technique. CEUS involves the use of microbubble contrast agents and specialised imaging techniques to show sensitive blood flow and tissue perfusion information. CEUS is safe with no requirement for ionising radiation and no risk for nephrotoxicity and can be easily performed. Ultrasound contrast agents consist of a solution of gas-filled shell-stabilised microbubbles with a diameter in the order of micrometres. These bubbles stay inside the blood pool and travel through all blood vessels, including the microvasculature [23].

Two studies have been performed with CEUS in IRE ablated lesions. In one study, CEUS was performed in patients with unresectable malignant hepatic tumours. In the second study, CEUS was performed following IRE in chemotherapy-refractory liver metastases in patients who were no candidates for surgery or radiofrequency ablation. The CEUS data showed a clearly confined devascularised lesion, corresponding to the ablated area [24,25].

**Clinical pathway**

Patients will be admitted one day before the scheduled IRE procedure. The transperineal ultrasound-guided insertion of the IRE needles and the electroporation will be performed under general anaesthesia and muscle relaxants. Full paralysis during electroporation is needed to prevent patient motion due to the high-voltage pulses. The specified target area is ablated. All patients will be given a Foley catheter before the procedure as well as prophylactic antibiotics. The day following the
procedure, the Foley catheter will be removed and the patient will leave the hospital after successful voiding. If this voiding is unsuccessful, the Foley catheter will be reinserted for one more week and removed at the outpatient clinic.

Adverse events and QoL assessment
The device related, periprocedural and postprocedural adverse events will be measured using the NCI Common Terminology Criteria for Adverse Events (CTCAE). CTCAE is a descriptive terminology that can be utilised for reporting adverse events. CTCAE is widely accepted throughout the oncology community as the standard classification and severity grading scale for adverse events in cancer therapy clinical trials and other oncology settings.

A grading scale is provided for each adverse event term [26]. QoL will be assessed by a validated comprehensive instrument (EPIC) designed to evaluate patient functioning and symptoms after prostate cancer treatment. The IPSS urinary QoL score (0-5) will used for with low scores demonstrating good QoL.

Follow-up
Patients will be dismissed one day after the procedure, once PSA measurement, adverse event reporting and VAS scoring have been completed and when the clinical condition allows it. At one and four weeks post-IRE, the patients are physically examined. Uroflowmetry is obtained and the patient is asked to fill out each questionnaire again. Two weeks post-IRE, a consultation is scheduled over the telephone. At one, two and four weeks post-IRE adverse events will be recorded. In case of clear harm to the participants, defined as severe adverse events (grade 3; CTCAE V4.0) or futility of the study, the trial will be terminated in consultation with the interdepartmental monitors and the data safety monitoring board.

The histological examination of the prostate specimen from both participating centres will be performed at the department of pathology in the Academic Medical Center, Amsterdam. It has been hypothesised that the IRE ablation zone can be defined by using the 2D ultrasound images in combination with the planning software on the IRE device. Histological examination will include macroscopic inspection – overall appearance, size and weight. Serial whole mount sections of 3-5mm, perpendicular to the urethra, are followed by a cut surface of each slice and inspected macroscopically and documented by photography. Whole-mount slices from apex to base will be embedded in paraffin, 4µm thick sections will be cut and examined with haematoxylin and eosin staining. The boundaries of the ablation zone will be determined by light
microscopy and marked on the slides, using the ultrasound imaging as a template. The volume of tissue alteration will be determined by adding the areas, as calculated using planimetrical analysis in AMIRA software (FEI Visualization Sciences Group). The outcome of the histopathological examination will be communicated to the patient at 1 or 2 weeks follow-up.

The mpMRIs will be evaluated by an uroradiologist. The ablation zones will be precisely demarcated and 3D reconstructed using AMIRA software. Subsequently, histopathology and imaging has to be assessed to determine the accurateness of ablation zone detection by mpMRI and CEUS and in order to design a valid preplanning model for IRE in prostate cancer.

**Sample size and data analysis plan**

The sample size of 16 patients is based on comparable studies with similar study design. Beerlage et al. performed a phase II study with high-intensity focused ultrasound (HIFU) with a sample size of 20 followed by a published case-series of 14 patients [27]. The other studies using radiofrequency (n=14), transurethral ultrasound therapy (n=8), and cryotherapy (n=7) reported similar patient numbers [28-30]. Brausi et al. was amongst the first presenting results of an IRE pilot safety study in 11 low risk prostate cancer patients. No major complications occurred during the procedure. The hospital stay was 1 day for all patients. Follow-up was done at 14, 30, 90 and 180 days and 19 months with physical examination, PSA, IPSS and IIEF. The mean IPSS reduced from 9.5 to 7.7, 7, 6.1, 4.28 and 4, respectively and the mean IIEF went from 16.2 to 13.2, 10.5, 10.5, 11 and 17.3. During follow-up, 1 patient presented with an acute urinary retention and 3 had transient urge incontinence. Mean PSA was 3.5, 2.9, 3.3 and 3.12 ng/mL after 30, 90, 180 days and 19 months, respectively. Prostate biopsies of the ablated area were performed after 1 month with a mean of 25 [range 15-41] biopsies. Pathological report was negative in 8 of the 11 patients (73%) and showed coagulative necrosis, granulomatosis, fibrosis and hemosiderosis. Three patients had a persistent adenocarcinoma. Therefore, one patient underwent radical prostatectomy and 2 were retreated with IRE [31]. Patients will be assigned with a research code on consecutive order as they enter the study. During the study, no information that can be related to the patient is shown on study material.
Outcomes
The primary outcome is safety as measured by the composite of procedural device and postprocedural adverse events, measured with CTCAE, EPIC-score, IPSS or required catheterisation time and IIEF and efficacy of ablation determined by histological examination post-prostatectomy. Secondary outcomes will be patients’ procedure satisfaction measured by patient satisfaction questions (included in EPIC score), postprocedural pain management and VAS pain score, time to ambulation, length of hospital stay.

Device and Procedure
The AngioDynamics Inc. HVP-01 Electroporation System (also registered as the NanoKnife™ IRE System, Figure 1) consists of three components; a low energy direct current (LED-C) generator, needle electrodes and Accusync ECG trigger (Accusync, Milford, Connecticut, USA).

Figure 1. Low energy direct current electroporation system (NanoKnife™ IRE System AngioDynamics).

The trigger was used to supply the pulses at a cardiac autosynchronous rate to decrease the risk of cardiac arrhythmias. The procedure will be performed under general anaesthetic and full paralysis using rocuronium (dose 1mg/kg) as well as a saddle block. The patients are placed in the extended lithotomy position and sterile draped and a transurethral catheter (16 Ch) is inserted (figure 2).
Figure 2. Patient is placed in extended lithotomy position with transperineally inserted electrodes using brachytherapy grid under ultrasound guidance.

The surgeon will assign the patients to two parallel groups. One group will have a focal ablation of the prostate; the other group will receive an extended ablation. In this way, we are able to assess the side effects of electroporation with different treatment scenarios. In grouping the patients, the tumour position will be taken into account monitored by preceding biopsies, MRI and CEUS. Therefore, mainly the tumour will be treated but also a part of surrounding healthy prostate tissue. The effects and the safety of the technique on both the tissues will be observed.

To define the treatment area, a biplane transrectal ultrasound system (Amsterdam Hi Vision Preirus, Hitachi Medical Systems, The Netherlands, equipped with an endocavity probe, type EUP-US33, C8.0-4.0, L10.0-5.0; Athens 2102 Falcon and 2202Pro Focus, BK Medical, Denmark, equipped with an endocavity probe models 8658 and 8848) will be used to visualise the prostate in both sagittal and axial direction. The volume and shape of the prostate will be determined. These data will be entered into the planning software system. One specified area will be chosen for ablation. Preferably, the side of the prostate is chosen where the major part of the tumour is located. Two to four 19-gauge unipolar electrode needles will be inserted transperineally using a brachytherapy grid under continuous ultrasound guidance (figures 3 and 4).
Figure 3. Three electrodes transperineally inserted through a brachytherapy grid.

Figure 4. Transrectal ultrasound with three inserted electrodes in right prostate lobe.
For an extended ablation with >4 electrodes, 2 electrodes will be repositioned followed by a second IRE course including the 4 electrodes in place. The locations will be verified using sagittal and axial ultrasound images of the prostate. Minimal distances between the needles and between the needles and essential structures (urethra, bladder neck, capsule and rectum) will be measured by ultrasound. The data will be transferred to the build-in planning software of the NanoKnife IRE device (figure 5).

![Figure 5. Planning software with localization of 2 needles (green numbered circles).](image)

The ablation procedure uses 90 pulses of 90 µs duration each with an electric field of 1500 V/cm between an electrode pair. Electric pulses are delivered between each of the electrode pairs. The actual treatment time will be approximately 5-10 minutes whereas the whole procedure is scheduled for 60 minutes.

**Ethics and dissemination**

Data will be presented at international conferences and published in peer-reviewed journals. The study is conducted and funded by the Clinical Research Office of the Endourological Society (CROES). The CROES is an official organ within the Endourological Society responsible for organizing, structuring and favoring a global network on endourological research. This research received no other specific grant from any funding agency in the public, commercial or not-for-profit sectors.
Benefits and harms
There are no benefits for the patients that participate in this study. Patients need to be informed about the risks of surgery and the procedure. Because the IRE technology is relatively new, there may be potential risks and side effects that are unknown at this time. A clinical risk analysis has been prepared which describes the hazards associated with the use of the device and the associated clinical risks associated with the procedure (e.g., general anaesthesia) along with the mitigations available to reduce this hazard.

Other accepted focal treatments for prostate cancer, such as percutaneous prostate cryoablation, have limitations such as variable damage at the lesion margins, injury to adjacent structures such as the rectum, urethra and neurovascular bundle, and long procedure times. These characteristics have limited the widespread acceptance of this modality despite certain demonstrated advantages over the more traditional treatments of radiation and RP. Research has been shown to have significant advantages in ablating hepatic tissue, such as rapid lesion creation, rapid lesion resolution, sparing of structures such as vessels and bile ducts, and uniform destruction throughout the IRE lesion [11]. It is theorised that these advantages will also apply to use in the prostate.

Data and Safety Monitoring Board
The Data and Safety Monitoring Board (DSMB) will act in an independent, expert and advisory capacity to monitor participant safety, and evaluate the efficacy and the overall conduct of the study. The responsibilities of the DSMB are to monitor safety data on a regular basis and, if required, on ad hoc basis to guide recommendation for continuation of the study or early termination because of clear harm. Furthermore to monitor efficacy data on a regular basis to guide recommendations for continuation of the study or early termination because of clear harm or futility, and to evaluate the overall conduct of the trial, including (1) monitoring of compliance with the protocol by participants and investigators; (2) monitoring of recruitment figures and losses to follow-up; (3) monitoring planned sample size assumptions; (4) reports on data quality; (5) reports on completeness of data; and (6) monitoring of continuing appropriateness of patient information.

Compensation for injury
The investigator has a liability insurance that is in accordance with article 7, subsection 6 of the WMO. This insurance is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for
Clinical Research in Humans of 23 June 2003). This insurance provides cover for damage to research participants through injury or death caused by the study (€450,000 for death or injury for each participant who participates in the research; €3,500,000 for death or injury for all participants who participate in the research; €5,000,000 for the total damage incurred by the organization for all damage disclosed by scientific research for the investigator in the meaning of said Act in each year of insurance coverage).

**DISCUSSION**

This will be the largest study cohort on IRE pre-RP. Previously, Neal et al. described two human IRE cases performed without serious adverse events followed by uncomplicated radical prostatectomies. The histology shows tissue necrosis plus a variable extent of reactive stromal fibrosis, inflammatory infiltrates and regenerative changes in epithelial lining of prostatic ducts [32]. So far, several focal therapies have been proposed and investigated for prostate cancer. In the past, the first phase I/II assessments of new focal ablation modalities have often been skipped and these methods were immediately used in clinical practice. The primary goals of this project are to determine the safety and efficacy of IRE. The interest in QoL is increasingly important, and it is therefore of paramount importance to assess this aspect in addition to adverse events, pain and side effects. Furthermore, it is essential to select validated and recommended questionnaires, to be able to compare the focal therapy trial outcomes [13]. An additional aspect of this study is the short-term evaluation of ‘salvage RP’, which is important, because some patients will fail focal therapy and will need secondary radical resection of the prostate.

This study protocol has some limitations. First, ablations of the posterior peripheral zone directly adjacent to the rectum have to be avoided in order to minimize possible rectal damage. Hydrodissection of the Denonvillers space may be an option to be able to ablate closer to the capsule of this zone in case of later projects evaluating the role of IRE as focal treatment.

Second, the maximum period between IRE and RP in this trial is 4 weeks. This limits analysis of the histopathology beyond this timeframe. It is clear that postponing the RP for evolving IRE effects is ethically not feasible. We will not use a customized mpMRI-based 3D-printed prostatectomy mold as described by Turkbey et al. Consequently MRI-slices and histopathology slides will not precisely match which will impede the exact comparison, resulting in a reduction of the correlation [22].
CONCLUSIONS

This trial will investigate the safety and efficacy of IRE in prostate cancer. Safety will be evaluated by reporting adverse events, side effects and QoL using validated questionnaires. Histopathology and radiological imaging will assess efficacy. The outcomes of this study will be used to develop a multi-centre single-blind randomised clinical trial (NCT01835977).
REFERENCES


Chapter 5

MRI and contrast-enhanced ultrasound imaging for evaluation of focal irreversible electroporation treatment: Results from a phase I-II study in patients undergoing IRE followed by radical prostatectomy

W van den Bos, DM de Bruin, A van Randen, MRW Engelbrecht, AW Postema, BG Muller, IM Varkarakis, A Skolarikos, CD Savci-Heijink, RR Jurhill, PJ Zondervan, MP Laguna Pes, H Wijkstra, TM de Reijke, JJMCH de la Rosette

Published in European Radiology 2015 Oct 8. [Epub ahead of print]
ABSTRACT

Objectives Irreversible electroporation (IRE) is an ablative therapy with a low side-effect profile in prostate cancer. The objective was: 1. To compare the volumetric IRE ablation zone on grey-scale transrectal ultrasound (TRUS), contrast-enhanced ultrasound (CEUS) and multiparametric MRI (mpMRI) with histopathology findings. 2. To determine a reliable imaging modality to visualize the IRE ablation effects accurately.

Methods A prospective phase I-II study was performed in 16 patients scheduled for radical prostatectomy (RP). IRE of the prostate was performed four weeks before RP. Prior to, and four weeks after the IRE treatment, imaging was performed by TRUS, CEUS, and mpMRI. 3D-analysis of the ablation volumes on imaging and on H&E-stained whole-mount sections was performed. The volumes were compared and the correlation was calculated.

Results Evaluation of the imaging demonstrated that with T2-weighted MRI, dynamic contrast enhanced (DCE) MRI, and CEUS, effects of IRE are visible. T2MRI and CEUS closely match the volumes on histopathology (Pearson correlation r 0.88 resp. 0.80). However, IRE is not visible with TRUS.

Conclusions mpMRI and CEUS are appropriate for assessing IRE effects and are the most feasible imaging modalities to visualize IRE ablation zone. The imaging is concordant with results of histopathological examination.
INTRODUCTION

Prostate cancer (Pca) is the most prevalent cancer in males in the Western world and the second most common cause of cancer death. Nevertheless, the majority of the patients will either have their cancer successfully cured or will die with rather than because of prostate cancer [1]. Since the liberal use of prostate-specific antigen tests and Pca screening began, the incidence of Pca diagnosis has increased substantially. Pca is traditionally managed by active surveillance or radical treatments, including radical prostatectomy and radiation therapy [2]. These radical methods often cause concomitant damage to prostate adjacent tissues, resulting in side-effects which include urinary incontinence (9·4-18·3%), impotence (40·9-95%) and bowel complications (21·9-35·8%) [3]. Nowadays, the concept of ablative therapy emerges as an approach positioned between expectant management and radical therapy in order to provide effective treatment while minimizing morbidity [4, 5]. In a select group of patients with localized low- and intermediate-risk Pca, ablative therapy might be a more suitable therapeutic option than total gland treatment. The clear purpose is to reduce the toxicity of organ-confined Pca treatments while adequately treating the cancer.

Irreversible electroporation (IRE) is an ablative technology that uses high voltage, low energy direct current, travelling between at least two electrodes. The electric current causes pore formation in the cell membrane that leads to permanent defects which results in cell death [6]. Literature on IRE reports advantages by sparing neighbouring vital structures such as the urethra, blood vessels and nerves [7, 8]. These properties could help to reduce, or even avoid, side effects.

For focal therapy, high quality imaging is of paramount importance for several reasons. The tumour should be identified and extracapsular extension should be excluded. During treatment, the urologist should be confident that the identified tumour area is completely ablated and no significant volume of tumour resides outside the targeted zone. During follow-up, recurrences or residual tumour should be recognized [9]. No literature is available on the role of imaging in follow-up of IRE in prostates. It is unclear what is to expect as image within state-of-the-art imaging modalities like greyscale transrectal ultrasound (TRUS), contrast-enhanced ultrasound (CEUS) or any multiparametric MRI (mpMRI) modality. We hypothesize that 1) TRUS, CEUS and mpMRI are feasible to identify the focal IRE ablation zone in 3D and 2) ablation zones identified on imaging are comparable to histopathology.

The aim of this study is to identify appropriate methods for the assessment of the IRE treatment zone and to determine which imaging modality (TRUS, CEUS or mpMRI) is feasible to visualize accurately the focal IRE ablation effect in 3D.
MATERIALS AND METHODS

A prospective study was conducted in sixteen patients with confirmed organ-confined prostate cancer who were scheduled for radical prostatectomy (RP). Inclusion and exclusion criteria were listed in Table 1.

Table 1. Inclusion and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>Patients with prostate cancer with indication for radical prostatectomy</td>
</tr>
<tr>
<td>Life expectancy &gt; 10 years</td>
</tr>
<tr>
<td>Able to visualize prostate gland adequately on transrectal US imaging</td>
</tr>
<tr>
<td>No prostate calcification greater than 5 mm</td>
</tr>
<tr>
<td>Ability of subject to stop anticoagulant and anti-platelet therapy for 7 days prior and 7 days post procedure</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding disorders</td>
</tr>
<tr>
<td>Active urinary tract infection</td>
</tr>
<tr>
<td>History of bladder neck contracture</td>
</tr>
<tr>
<td>Inflammatory bowel diseases</td>
</tr>
<tr>
<td>Concurrent major debilitating illness</td>
</tr>
<tr>
<td>ICD / Pacemaker /Cardiac History</td>
</tr>
<tr>
<td>Prior or concurrent malignancy</td>
</tr>
<tr>
<td>Biologic therapy for prostate cancer</td>
</tr>
<tr>
<td>Chemotherapy for prostate cancer</td>
</tr>
<tr>
<td>Hormonal therapy for prostate cancer within 3 months of procedure</td>
</tr>
<tr>
<td>Radiotherapy for prostate cancer</td>
</tr>
<tr>
<td>Transurethral prostatectomy or urethral stent</td>
</tr>
<tr>
<td>Prior major rectal surgery</td>
</tr>
</tbody>
</table>

The patients underwent a focal IRE procedure approximately one month prior to the surgery. mpMRI (by means of T2-weighted imaging (T2W), dynamic contrast-enhanced imaging (DCE) and diffusion-weighted imaging (DWI)), TRUS, and CEUS imaging were performed prior to the IRE procedure and approximately four weeks after the IRE treatment, on the day of admission for the RP. Patient characteristics are presented in Table 2.
The study was conducted in two participating centers: AMC University hospital Amsterdam and Sismanoglio University hospital Athens. The study was approved by the two individual research ethics committees and registered in the clinicaltrials.gov database (NCT01790451). The trial was executed according to the study protocol previously reported in Van den Bos et al. [10].

**IRE**

The IRE procedures were performed using Nanoknife® IRE system (AngioDynamics Inc, Queensbury, NY) under general anesthesia and full paralysis. The electrodes were placed transperineally in the prostate under ultrasound guidance (Figure 1), delivering 90 pulses of 90 µs duration each with an electric field of on average 1500 Volt (V) per cm distance between the electrode (voltage-to-distance ratio).

The standard setting of 1500 V/cm was adapted when the current showed a constant low amperage or high amperage ranging from 1200 to 2100 V/cm (Table 3).
Figure 1. Patient positioned in high-lithotomy position with three ultrasound-guided transperineally inserted electrodes.
It was the initial experience of IRE in both participating centers. The number and configuration of the electrodes were varied to assess the effect on the ablation area. The electrodes were placed in the lobe found most positive biopsies. Therefore, exact targeting of the tumor was not pursued.

In the first patient, the IRE procedure was performed using two electrodes. Five patients were treated with three electrodes and ten patients with four electrodes or more. In patient 6, a pullback of 15 mm of the electrodes was performed after the first ablation cycle. In patient 8, the two lateral electrodes were repositioned after 1 ablation cycle to the other lobe, in order to perform a bilateral ablation. Additional details about the device and procedures were described in BMJ Open [10].

**Greyscale transrectal ultrasound**

Greyscale TRUS scanning was performed in all 16 patients before the IRE procedure and four weeks after IRE. Patients were scanned in Amsterdam using a Philips IU22 Ultrasound system with an end-firing transrectal probe (Phillips Healthcare, Bothell, WA, USA) and in Athens using a Falcon 2101 EXCL (BK Medical, MA, USA) while positioned in the left lateral decubitus position. Greyscale TRUS standard volumetry was performed, followed by three sweeps for later 3D reconstruction consisting of a transversal sweep from base to apex, a longitudinal sweep from base to apex, and a longitudinal sweep from left to right.
**Contrast-enhanced ultrasound**

Subsequently, CEUS recordings were acquired before and four weeks after IRE in the 12 patients treated in Amsterdam. CEUS imaging was performed with the same Philips IU22 machine using the protocol as described by Kuenen et al.\(^1\) with an ultrasound contrast agent that consists of gas-filled microbubbles, which are stabilised by a protein or lipid shell (Sonovue®, Bracco, Milan, Italy). The prostate was scanned in four planes: base, base-mid, mid-apex, and apex. For each plane, recordings were completed after a bolus injection 2.4 mL of UCA followed by a 5 mL saline flush. Subsequently, transversal sweeps from base to apex were performed to allow calculation of the dimensions of the contrast deficits after IRE.

**Multiparametric MRI**

Two mpMRI studies were performed per patient. The first was acquired prior to the IRE procedure and the second was performed, four weeks after the IRE treatment and one day prior to RP. The mpMRIs were made according to the ESUR guidelines \(^2\). All patients included in the AMC Amsterdam (n=12 patients) underwent mpMRI performed on a 1.5 Tesla AVANTO® MRI scanner (Siemens Healthcare, Erlangen, Germany) using an integrated endorectal-pelvic phased-array coil (Medrad, Warrendale, USA) \(^3\). The mpMRI protocol in Athens (n=4 patients) was performed on a 3·0 tesla MAGNETOM Trio (Siemens, Healthcare, Erlangen, Germany) with a pelvic phased-array coil. In the AMC; first, T2 weighted turbospin echo sequences were performed in axial, coronal, and sagittal planes, covering the prostate and seminal vesicles. Second, a single-shot-echo-planar imaging sequence with diffusion module and fat suppression pulse was implemented. ADC-maps were automatically calculated by the scanner software using all 3 b-values. Next dynamic gadolinium enhanced MRI was performed using fast gradient echo sequences. Seven slices covering the prostate were acquired with a temporal resolution of 7 slices per 2.1 seconds before and 90 seconds after intravenous administration of 0.1 mmol of gadopentetate dimeglumine per Kg of body weight Gadolinium DTPA (Gadovist). To supress bowel peristalsis, intravenous or intramuscular administration of 20mg Buscopan® was given. In Athens at 3T, first T2 weighted turbospin echo sequences were performed in transverse, coronal and sagittal direction. Next single-shot-echo-planar imaging sequences with diffusion module and fat suppression pulse was implemented. ADC-maps were automatically calculated by the scanner software using two b-values. Finally dynamic contrast enhanced MRI was performed using fast gradient echo sequences with a similar temporal resolution as in the AMC.
3D assessment of imaging
The pre- and post-treatment images of the prostates were examined in consensus by two uro-radiologists of the AMC, blinded to pathology results. Cross-sectional images from TRUS, CEUS, and mpMRI were visually inspected on lesion boundaries and contrast differences. The ablation zones were volumetrically evaluated in CEUS and T2 MRI data. For MRI, the ablation zones were manually segmented frame by frame using a delineation tool using Amira®. For CEUS, the ablation zones were automatically segmented in 3D using averaging, window leveling and wand tracing in ImageJ/FIJI [14], or manually frame by frame using a delineation tool. The precisely outlined areas were segmented and aligned to create a 3D reconstruction of the ablation zones and the volumes were determined using AMIRA shape-analysis package software (Version 5.5; FEI Visualization Sciences Group, Mérignac Cédex, France).

Histopathology
To ensure consistency of histopathological analysis, the examination of the prostate specimens from both centers was performed at the pathology department of the AMC. The serial sections of 3-5 mm were cut perpendicular to the rectum according to the axial MRI plane, followed by a cut surface of each slice and inspected macroscopically and documented by photography. Whole-mount slices from apex to base were embedded in paraffin; 4 µm thick sections were cut and examined with H&E staining. The boundaries of the ablation zone were determined manually using light microscopy and marked on the slides. The outlined slides were scanned with IntelliSite Ultra-Fast Scanner (Philips, Best, the Netherlands) and the volume of tissue alterations were defined by segmentation as indicated by the pathologist followed by volumetric shape-analysis in AMIRA. The acquired volumes were adjusted for shrinkage during fixation using the pre-fixation prostate dimensions divided by the post-fixation volume.

Data-analysis
All extracted volumes of CEUS, T2 MRI and PA were directly compared and visualized using an x/y scatterplot. The scatter data was linearly fitted from which a Pearson correlation value (goodness of fit, r) and slope was calculated as a measure of correlation. All the images were matched with the PA-volumes. To calculate the error per 3D reconstruction for CEUS, T2 MRI and PA, the lesion volume was divided by the number of slices from which the 3D dataset was constructed.
RESULTS

Procedural results
No serious adverse event was observed during the procedure or hospital stay. All patients, except for one due to social reasons, were discharged from the hospital one day after the IRE treatment.

Qualitative evaluation of imaging
Qualitative evaluations of the imaging modalities showed that grey-scale TRUS inadequately visualized IRE ablation effects in all sixteen cases. All post-IRE CEUS provided a clear dynamic homogeneous non-perfused image of the ablation zone in all patients, except for the first treated patient, in which no non-perfused area could be detected. In 9 out of 12 scans, the dataset was sufficient to reconstruct the ablation zone in 3D. On T1-dynamic scans, the IRE effects are identifiable as areas of non-contrast enhancement. On T2W images, fifteen patients showed heterogeneous signal intensity and nine patients showed marked hypo-intense margins suggestive of fibrosis. The mpMRI of the first patient did not show a clear ablation zone. Three of the patients had a slightly higher intralesional T2-signal intensity, possibly because of oedema. Twelve patients had high signal intensity focal intralesional areas due to haemorrhage on the pre-contrast T1-weighted images. TRUS and CEUS images were simultaneously acquired and are therefore automatically matched. mpMRI images were visually matched to the CEUS data (Figure 2). 3D reconstructions are shown in Figure 3.
Figure 2. Imaging modalities pre-IRE (left) and four weeks post-IRE (right): a. Grey-scale ultrasound; cannot image the ablation zone b. CEUS; shows a sharp-demarcated dark area presenting non-perfused tissue c. DCE-MRI shows a sharp-delineated non-enhancing lesion d. T2-weighted MRI shows hypo intense lesion.
Qualitative evaluation of histopathology

Macroscopic assessment showed, in 15 cases, lesions in the treated zone with a central white necrotic zone surrounded by an outer, dark red haemorrhagic zone (Figure 4). Microscopic assessment showed, in the same 15 cases, fibrotic and necrotic areas with mild inflammation with sharp demarcations. The prostate specimen of the first treated patient contained solely fibrotic tissue in a bilateral pattern without any necrotic component, not showing any relation to the unilateral electrode configuration. This suggests that the fibrosis was pre-existent and not caused by IRE, and therefore excluded for analysis.
Quantitative evaluation

**mpMRI – PA correlation**

Comparison of the ablation zones volumes traced on the post-IRE T2-weighted MR images with the ablated volume traced on H&E stained PA images revealed that MRI-measured volumes were 1.16 times PA measurements. The Pearson correlation index between the two sets of measurements was $r = 0.88$ with a slope of 0.75 (Figure 5A).

**CEUS – PA correlation**

Ablation zone volumes traced on the post-IRE CEUS images compared with volumes traced on H&E PA images found that, on average, CEUS volumes was 1.57 times greater than PA measurements. The Pearson correlation index between these sets of measurements was $r = 0.80$ with a slope of 0.82 (Figure 5B). The errors on CEUS values were too small to display in the graph.
Figure 5. Correlation of ablation volumes T2-weighted MRI and H&E pathology (A). The Pearson correlation index between the two sets of measurements was $r = 0.88$ with a slope of 0.75. Correlation of ablation volumes CEUS and H&E pathology (B). The Pearson correlation index between these sets of measurements is $r = 0.80$ with a slope of 0.82. The errors on CEUS values are too small to display in the graph.

**DISCUSSION**

This is the first study assessing three different imaging modalities pre- and post-IRE treatment for prostate cancer. This study demonstrated that either mpMRI or CEUS can be used to accurately and reliably follow the area treated with IRE. Lindner et al. published a similar designed case-study with focal laser ablation [15]. Comparison of the ablated volume traced on H&E stained PA with the volumes traced on MRI revealed on average that MRI-measured volume was 1.4 times PA measurements with a Pearson correlation index of $r = 0.79$. Grampsas et al. published a comparable trial with cryoablation concluding that TRUS overestimates the area of prostatic tissue destroyed by the ablation and this challenges the assumption that the entire prostate is lethally frozen when its boundaries are included within the hypoechoic ice ball observed on TRUS [16]. Several studies have been performed using high-intensity focused ultrasound (HIFU) followed by RP. Madersbacher et al. demonstrated that HIFU induced sharply delineated intraprostatic coagulative necrosis within the target area, whereas alterations of periprostatic structures were never observed [17]. The histopathological effects of IRE in correlation to treatment planning and probe placement, in addition to effects to the prostate capsule, urethra and neurovascular bundle are recently described by Van den Bos et al [18]. Beerlage et al. showed PA reports of radical prostatectomy specimens following HIFU treatment with marked and complete necrosis in the treated area [19, 20]. None of these studies include post-treatment imaging assessing the ablation effects during follow-up.
A limitation of the present study is the low number of CEUS datasets, available for analysis. Because CEUS could only be obtained in Amsterdam, CEUS analysis was done using the data of twelve patients. In nine patients, 3D-reconstructions could be made using the CEUS data. In the remaining four scans the transversal sweep was insufficient for a reliable 3D-analysis. Wiggerman et al. performed CEUS immediately after and 20 minutes after IRE of hepatic tumours and concluded that CEUS allowed good prediction of ablation result following IRE [21]. Furthermore, at this early phase of research, the IRE treatments were performed without curative intent. So within the trial, the efficacy for treatment of prostate cancer could not yet be assessed. Another limitation is that the fixated prostates were cut by hand, possibly leading to reduction of the correlation between the imaging and PA.

In the analysis of the PA, the prostate specimen of the first patient (treated with a two electrode-configuration) showed confluent fibrotic tissue without a necrotic component, in contrast to all other specimens which contained a necrotic focus. Additionally, the IRE treatment was performed unilaterally yet, the fibrosis was present in both lobes and therefore considered as pre-existent, as associated with chronic inflammation and aging. Together with the additional absence of in-field necrotic tissue, the patient was excluded from further analysis. However, Neal et al.[22] also performed IRE treatments using only two electrodes in prostate cancer patients (n=2) that did show PA ablation zones of 1.14 cm$^3$ and 2.46 cm$^3$. This may indicate that the incomplete ablation was not due to the low number of used electrodes and the cause of the required exclusion of this patient remains unknown.

The close matches of T2MRI, DCE-MRI and CEUS with PA confirm the accuracy of these imaging modalities in rendering the ablation zone. The latter two modalities are based on tissue perfusion and both modalities clearly show a loss of enhancement what represents devascularisation and thus tissue destruction. Since the PA showed mainly end-stage fibrotic tissue, the destruction of tissue four weeks post-IRE is reasonably representing the long-term effects. So, ablation effects may be reasonably measurable by these imaging modalities.

**CONCLUSIONS**

T2MRI, and CEUS are feasible imaging modalities to visualize the IRE ablation effects in 3D with a strong Pearson’s correlation of $r = 0.88$ and $r = 0.80$, respectively compared with histopathology. Grey-scale US is insufficient for assessing IRE ablation volumes. mpMRI and CEUS should be used in future research for the evaluation of IRE-effects ablation in focal therapy for prostate cancer.
REFERENCES


Chapter 6

The correlation between the electrode configuration and histopathology of irreversible electroporation ablations in prostate cancer patients

W van den Bos, DM de Bruin, RR Jurhill, CD Savci-Heijink, BG Muller, IM Varkarakis, A Skolarikos, PJ Zondervan, MP Laguna-Pes, H Wijkstra, TM de Reijke, JJMCH de la Rosette

Published in World Journal of Urology 2015; Aug 22. [Epub ahead of print]
ABSTRACT

Purpose Irreversible electroporation (IRE) is a novel minimally invasive therapy for prostate cancer using short electric pulses to ablate prostate tissue. The purpose of this study is to determine the IRE effects in prostate tissue and correlate electrode configuration with the histology of radical prostatectomy (RP) specimens. We hypothesize that the area within the electrode configuration is completely ablated and that the area within the electrode configuration is predictive for the ablated area after treatment.

Methods A prospective phase I-II study was conducted in 16 consecutive patients with histopathologically confirmed prostate cancer scheduled for RP. Focal or extended IRE treatment of the prostate was performed four weeks prior to RP. The locations of the electrodes were used to calculate the planned ablation zone. Following RP, the specimens were processed into whole-mount sections, histopathology (PA) was assessed and ablation zones were delineated. The area of the tissue alteration was determined by measuring the surface. The planned and the histological ablation zones were compared, analyzed per individual patient and per protocol (focal vs. extended).

Results All cells within the electrode configuration were completely ablated and consisted only of necrotic and fibrotic tissue without leaving any viable cells. The histological ablation zone was always larger than the electrodes configuration (2.9 times larger for the 3 electrodes configuration and 2.5 times larger for the ≥4 electrode configuration). These ablation effects extended beyond the prostatic capsule in the neurovascular bundle in 13 out of 15 cases.

Conclusions IRE in prostate cancer results in completely ablated, sharply demarcated lesions with a histological ablation zone beyond the electrode configuration. No skip lesions were observed within the electrode configuration.
INTRODUCTION

Prostate cancer has become the most prevalent cancer in men and forms a significant health risk in the Western world [1]. PSA tests are currently performed regularly and imaging techniques have improved, leading to a considerably increased detection of localized prostate cancer [1]. The current treatment options for localized prostate cancer are active surveillance and therapy with curative intent (surgery or radiotherapy), depending on the stage of the tumour and patients’ consideration. A variety of ablative therapies has been introduced for treatment of localized prostate cancer. It functions as middle ground between the traditional options. The rationale behind these so-called focal therapies is to target only the tumorous areas and leave healthy tissue and adjacent structures intact. Most used ablative technologies are cryotherapy, high-intensity focused ultrasound (HIFU) and radiotherapy [2]. A novel technique in the armamentarium is irreversible electroporation (IRE). It uses pulsed high voltage - low energy direct electric current for ablation. The electric energy is delivered through needle-electrodes, placed circumferential around the tumour zone. Cell-membrane potentials are disturbed by the consecutive high-voltage (usually around 1500 V/cm) electric pulse trains between at least two spatially separated electrodes, which leads to irreversible permeability of cell membranes and results in apoptotic cell death [3]. Literature on IRE reports advantages as sparing surrounding vital structures including blood vessels and connective tissue [4]. By sparing these structures unharmed, patients might maintain their potency and continence. Successfully treating prostate cancer requires an accurate prediction of the treatment zone through treatment planning. Planning of IRE is currently based on mathematical and numerical models in multicellular tissue models and animals studies [5–7]. The aim of this study was to assess the IRE ablation zone in specimens from radical prostatectomies by using different ablation protocols and to correlate the cross-sectional ablation zone on histopathology with the electrode configuration. We hypothesize that 1) the area within the electrode configuration is completely ablated; 2) the area within the electrode configuration is predictive for the cross-sectional ablated area after treatment, irrespective to the used ablation protocol.
METHODS

Setting
Sixteen patients with confirmed localized prostate cancer, scheduled for a radical prostatectomy, were enrolled between August 2013 and April 2014 to undergo an IRE treatment approximately 4 weeks prior to RP. The patients’ mean age was 60 years (range 44 - 75) and had an average serum prostate specific antigen (PSA) concentration of 9.5 µg/L (range 4.4 - 22.5). Patient’s characteristics are shown in Table 1. Twelve patients were treated at the university hospital in Amsterdam and four patients in the university hospital in Athens. The institutional review boards of both hospitals approved the study. Written informed consent was obtained from every patient.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patients (N = 16)</th>
<th>Age (years)</th>
<th>60 (range 44-75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PSA (µg/L)</td>
<td>9 (range 3.6 – 25)</td>
<td></td>
</tr>
<tr>
<td>Prostate volume (mL)</td>
<td>39 (range 19 – 60)</td>
<td></td>
</tr>
<tr>
<td>Gleason score (TRUS-guided biopsy)</td>
<td>3 + 3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>3 + 4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4 + 3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4 + 4</td>
<td>2</td>
</tr>
<tr>
<td>Disease distribution (TRUS-guided biopsy)</td>
<td>Unilateral</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Bilateral</td>
<td>5</td>
</tr>
<tr>
<td>Gleason score (RP specimen)</td>
<td>No tumour present</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3 + 3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>3 + 4</td>
<td>6</td>
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<tr>
<td></td>
<td>4 + 3</td>
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</tr>
<tr>
<td></td>
<td>3 + 5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4 + 4</td>
<td>0</td>
</tr>
<tr>
<td>Disease distribution (RP specimen)</td>
<td>Unilateral</td>
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<tr>
<td></td>
<td>Bilateral</td>
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<td>Tumour stage</td>
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<td>pT2c</td>
<td>11</td>
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<tr>
<td></td>
<td>pT3a</td>
<td>4</td>
</tr>
</tbody>
</table>
IRE procedure

The IRE procedure was performed using transperineally inserted electrodes through a brachytherapy grid under continuous ultrasound guidance. The location of each electrode and the distances between the electrode pairs were assessed on ultrasound and used as prediction of the ablation zone.

The IRE-treatment was performed using the Nanoknife® (AngioDynamics Inc., Queensbury, NY) (figure 1). A low energy direct current generator delivering high voltage-pulses by 19-gauge monopolar electrode needles. The procedures are performed under general anesthesia. An electrocardiogram (ECG)-gating device (Accusync, Milford, Connecticut) was used to synchronize pulse delivery within the refractory period of the heart to prevent cardiac arrhythmias. Rocoronium was intravenously administered to achieve adequate muscle relaxation and was confirmed by twitch absence using a peripheral nerve stimulation test.

Figure 1. Patient in lithotomy position with three transperineally inserted electrodes under ultrasound guidance
The IRE treatment was performed following a focal or extended ablation protocol. To achieve focal prostate ablation, one 2- and five 3-electrodes configurations were performed. Ten extended ablations were performed using four or more electrodes. The active exposure length of the electrodes was set on 15 mm. The electrodes delivered 90 pulses of 90 µsec duration each between every pair, with a pulse intensity set at 1500 V/cm (voltage-to-distance ratio). A current of 20 to 40 A runs during the pulse. The standard setting of 1500 V per cm distance between the needles was adapted when the current showed low amperage or high amperage, with a maximum of 3000 V. All electrodes were inserted at a distance of at least 5 mm from the rectal wall. In one patient, a pullback of 15 mm of all four electrodes was performed after the first ablation to increase the length of the ablation zone. During another procedure of a four-electrode configuration, the two lateral electrodes were repositioned after 1 ablation cycle to the other lobe, (covering the urethra) in order to perform a bilateral ablation. At this phase of research, the IRE treatment was not performed with curative intent. The electrodes were inserted in a lobe found positive by biopsy, however exact targeting of the tumour was not pursued. The procedures and IRE device are described in detail in van den Bos et al. [8].

Treatment prediction

The area within the electrode configuration was determined by the locations of the inserted electrodes as measured on the ultrasound images as indicated in figure 2a (focal ablation protocol) and in figure 3a (extended ablation protocol). The area within the configuration was delineated (figures 2b/3b). Ultrasound imaging was scaled using the grid on the images and the areas within the electrodes were determined using image-analysis software ImageJ/FIJI. These areas were used as prediction of the ablation zone.
Figure 2. a. Ultrasound image showing the three inserted electrodes. b. The area within the electrode configuration is delineated. c. H&E slide with the outlined ablation zone. d. The ablation zone is delineated.
Figure 3. a. Ultrasound image showing the four inserted electrodes. b. The area within the electrode configuration is delineated. c. H&E slide with the outlined ablation zone. d. The ablation zone is delineated.

**Histology assessment**

Macroscopic assessment and processing included measurement of size and weight, and orientation was achieved by using multiple colours for different planes. After formalin fixation the prostate was cut into serial sections of 3 - 5 mm thickness, perpendicularly to the urethra. The tissue was embedded in paraffin after which 4 µm thick sections were cut and examined with an H&E stain. The characteristics of both the ablation zone (PA) and non-ablated zone were determined by light microscopy. The tissue is considered affected by IRE if the cells showed necrosis and/
or denudation in case of epithelial tissue and necrosis and denudation are easily seen on an H&E slide. The margins of the ablation zone and tumour zones were delineated on the slides (figures 2c/3c and 2d/3d). Furthermore, any affected essential structure (urethra, capsule, neurovascular bundle) was determined per patient and scored by the uropathologists. To assess correlation of the distance between electrodes and essential structures were measured in mm using ultrasound images. Because of non-normal distributed, non-paired data, statistical analysis was performed using a two-tailed Mann-Whitney test.

Histology ablation areas
The edited slides were scanned 40X with the IntelliSite Ultra-Fast Scanner (Philips, Best, the Netherlands) and the areas of tissue alterations were determined by segmentation as designated by the pathologists. Subsequently, the slide representing the centre of the ablation zone, containing the most expanded ablation, was determined. The precisely outlined areas were scaled using the dimensions of the microscope slide. The ablation areas were calculated with the use of ImageJ/Fiji. The extracted cross-sectional ablation zones were adjusted for shrinkage during fixation using the pre-fixation prostate dimension divided by the post-fixation dimension.

Electrode configuration and histology correlation
To assess if the electrode configuration correlates to PA, all the areas within the inserted electrodes (in cm²) were directly compared and matched with the cross-sectional histology areas.

Analysis of focal versus extended ablation zones
It was hypothesized that no difference would be assessed between the two ablation protocols in the comparison of the predicted and histological determined ablation areas. To test validity, the factorial difference between the predicted and histology area in cm² is plotted for the two protocols by dividing histology analysis by the electrode configuration.

RESULTS

IRE procedure
Six patients were treated using the focal ablation protocol. The extended protocol was performed in the remaining ten patients. All the procedures went uneventful and
no serious adverse events were observed during the procedure or hospital stay. None of the patients have been withdrawn because of adverse events. The voltage-to-distance ratios ranged from 1200 to 2100 V/cm with currents between 15 and 45 A. The procedure specifications per patient are shown in Table 2.

Table 2. Procedure specifications

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Number of electrodes</th>
<th>Voltage-to-distance ratio (V/cm)</th>
<th>Range amperage (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1500</td>
<td>15 - 18</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1500-1700</td>
<td>22 - 28</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1500</td>
<td>21 - 42</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1200-1500</td>
<td>22 - 45</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>1500-1800</td>
<td>26 - 35</td>
</tr>
<tr>
<td>6</td>
<td>4 + pullback</td>
<td>1350-1500</td>
<td>25 - 45</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1500-1650</td>
<td>22 - 28</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>1350-1500</td>
<td>25 - 40</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>1500-1800</td>
<td>25 - 38</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>1500</td>
<td>23 - 35</td>
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<tr>
<td>11</td>
<td>3</td>
<td>1500-1800</td>
<td>21 - 31</td>
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<tr>
<td>12</td>
<td>4</td>
<td>1500-1650</td>
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<td>15</td>
<td>3</td>
<td>1500-2100</td>
<td>18 - 25</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>1500</td>
<td>22 - 32</td>
</tr>
</tbody>
</table>

Histology assessment

Gross examination showed sharply demarcated haemorrhagic lesions at the ablation site in all cases, except for the specimen of patient one where only two electrodes were used. Microscopic assessment showed hemorrhagic, necrotic and fibrotic areas in all cases, again except for patient one. In the first patient the specimen contained only fibrotic tissue with a bilateral extension, without any relation to the unilaterally inserted electrodes. This patient was the only one treated with two electrodes with a low current output (15-18 A), what is considered as insufficient by the manufacturer. Therefore the case was excluded from further histological analysis. All other prostate specimens showed sharply delineated ablation zones with completely non-viable tissue consisting of necrotic and fibrotic tissue within the electrode configuration. The IRE effects comprised areas ranged from 5 to 40% of the total prostate tissue.

Electrode configuration – histology association

The areas traced within the electrode configuration (in mm²) were compared with the area on the H&E PA image and are displayed per patient in figure 4a for the focal ablation protocol and in 4b for the extended ablation protocol.
Figure 4. a. The areas within the electrode configuration and the cross-sectional areas of the histology analysis of the focal ablation protocol, presented per patient. The results outlined in green are from the same patient as displayed in Figure 2. b. The areas within the electrode configuration and the cross-sectional areas of the histology analysis of the extended ablation protocol, presented per patient. The results outlined in green are from the same patient as displayed in Figure 3.

The results, grouped per protocol, are shown in figure 5.

Figure 5. a. Boxplots showing the results of the areas electrode configuration of the focal ablation protocol and the outcomes of the histology analysis. b. Boxplots showing the results of the areas electrode configuration of the extended ablation protocol and the outcomes of the histology analysis.

None of the patients had skip lesions within the configuration of the electrodes. When using the focal ablation protocol, the histological analysis showed ablation zones of 2.9 times (range 1.3 - 4.0) greater than the area of the electrode configuration. Using the extended ablation protocol, the histological analysis showed ablation zones of 2.5 times (range 1.1 - 4.3) greater than the electrode configuration. Patient 4 in the
focal protocol and patient 4 and 6 in the extended protocol appeared as outliers, with a relatively small ablation area. These variant outcomes could not be explained by the prostate tissue composition including the presence of glandular hyperplasia, inflammation, atrophy, Gleason score or prostate size. The factorial outcomes were presented per ablation protocol in figure 6. No significant difference was found between these outcomes.

IRE effects were observed extending beyond the prostatic capsule in twelve cases and in the neurovascular bundle in thirteen of the fifteen prostates. The urethra was affected due to IRE treatment in nine prostates. Chronic inflammation varied from mild to moderate with only one cases showing focal severe inflammation. Statistical analysis of affected versus unaffected tissue structures revealed no difference between structures close to or at distance to the inserted electrodes (urethra P-value 0.47, IQR = 5.8 – 9.6, prostatic capsule P-value 1.0 IQR = 5.0 – 6.4), neurovascular bundle P-value = 0.38, IQR 4.9 – 6.3) as shown in figure 7.
Figure 7. Boxplots showing of damaged and non-damaged essential structures revealing no difference between structures close to and more distant to the inserted electrodes (urethra P-value 0.56, min-max = 4.8-12, prostatic capsule P-value 0.94, min-max = 1.4–11, neurovascular bundle P-value 0.58, min-max = 5.6-25.3).

**DISCUSSION**

The present study was designed to determine if IRE was able to achieve complete cell death. It is crucial for ablative therapies to secure that the targeted zone is entirely ablated, without leaving any viable cells. In this study, the histology outcomes confirm that IRE effectively ablates all prostate tissue enclosed within the area of the inserted electrodes. The first examined viable cells were localised at the borders of the ablation zone. All inserted electrodes were placed with at least 5 mm distance to the rectal wall. During follow-up, no clinically significant adverse events concerning rectal wall damage occurred. The evaluation of the exact safe margin to be kept from the rectum was beyond the scope of this trial, however in four instances a margin between 4.5 to 6.0 mm was kept with no subsequent adverse events.

Similar studies performing ablative therapies followed by RP show various results. Pisters et al. [9] did not achieve complete tumour destruction with whole-gland cryotherapy in three of the seven patients. Grampsas et al. [10] reported the potential for salvage radical prostatectomy in biopsy-proven recurrences of the 62 evaluated cases treated with complete cryoablation. Six of these patients underwent a salvage radical prostatectomy showing necrotic tissue in the area of probe-placement with a sharp border demarcating these regions from the adjacent viable oedematous connective tissue. The whole-mount sections of the prostate specimen revealed residual prostate cancer after supposed successful cryoablation. Two comparable studies were performed to evaluate the histological impact and efficacy of transrectal HIFU. Beerlage et al. [11] showed incomplete necrosis at the dorsal side of
the prostate in all treated patients. In two of the nine cases, this area contained vital tumour tissue. Madersbacher et al. [12] achieved a successful ablation in three out of ten participating patients using HIFU. In the remaining seven patients, the tumour was partly targeted but persistent tumour tissue was found with histopathological examination. These studies showed that complete tissue destruction was not achieved in all participants. In contrast, our study using IRE ablation showed that within the targeted area all cells were destroyed. Several other papers, mostly based on canine studies, confirm our findings using the IRE technology (5,12,13). However, the sizes of the ablation areas in histology are much larger than the configuration, with a factor of 2.7 on average. An explanation is that the ablation zone follows the electrical field (E-field) and this expands beyond the electrodes like described in the mathematical model of irreversible electroporation by Edd et al [5]. Also potential thermal properties accompanying the IRE effects like described by Wagstaff et al. [13] may enlarge the ablation area.

Limitations of our study include possible shrinkage artefacts and the short term of follow up of four weeks. During these four weeks following the IRE procedure, the inflammatory and fibrotic processes resulting in necrosis causes deformation of the prostate gland. An additional limitation is the small sample size of sixteen patients. Therefore, the results may not be generalized to all prostate cancer patients. A successive randomized clinical trial covering the next phase has already started under the umbrella of the clinically research office of the endourological society (CROES) and aims to include 200 patients (NCT01835977). The study was also limited by the maximal follow-up of four weeks due to the need for RP in these patients. Although longer follow-up is desirable, postponing the scheduled RP was ethically no option. Actually, it is believed that the duration of the follow-up was sufficient since the histology showed necrotic and fibrotic elements implying the IRE effects were beyond the early effects of the treatment (inflammation) and before longer-term effects (fibrosis and repair). Interestingly, the first IRE treatment in our study was performed using a unilateral two-electrode configuration. Histological analysis did not show any necrotic area but diffuse bilateral fibrosis throughout the prostate. The reported current ranged from 15 to 18 A. Dunki-Jacobs et al. stated that the change in resistance and the slope of the resistance should be used to assess successful tumor ablation during IRE in pancreas [14]. The small change in current with an increase of 3 amperes of case 1 may imply an unsuccessful ablation. However, Neal et al. performed 5 IRE treatments using two electrodes (1 in canine prostate and 4 in human prostate) generating low currents and high currents and the specimens all showed an ablation zones with a necrotic focus.[15] Proper evaluation of an electrode configuration requires multiple
repetitions. Since there was only one case with a 2-electrode configuration and low current, the cause of the unsuccessful ablation remains unclear.
The findings of our study are important since it documents the targeted zone within the needles configuration where all cancer will be eradicated.

CONCLUSIONS

IRE in prostate cancer results in a completely ablated, sharply demarcated ablation areas without leaving any viable cell within the electrode configuration. The electrode configuration using the focal ablation protocol and the extended ablation protocol respectively results in a 2.9 and 2.5 times greater cross-sectional histological ablation zone.
REFERENCES

Chapter 7

Quality of life and safety outcomes following irreversible electroporation treatment for prostate cancer: Results from a phase I-II study

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Published in Journal of Cancer Science & Therapy 2015;10(7),312-321
ABSTRACT

Objective Prostate cancer treatment as radical prostatectomy or radiation therapy is associated with collateral tissue damage resulting in side-effects. Irreversible electroporation is a minimally invasive technique that has shown to be effective in destroying tumour cells and has been proposed to diminish the treatment related morbidity. The aim of the study was to evaluate the safety and quality of life (QoL) and functional outcomes of extended and focal irreversible electroporation (IRE) in prostate cancer.

Materials and methods IRE-ablations of the prostate were performed using two treatment protocols (focal and extended) to assess potential variation in outcomes. The safety of IRE was assessed by the device-related, periprocedural- and post procedural adverse events. Post-procedural quality of life was measured by prostate cancer-specific QoL questionnaires. Validated questionnaires were used to determine the following outcomes: genitourinary side effects, urinary and erectile function. Post-procedural pain was scored using the visual analogue scale (VAS) and the length of hospital stay was documented.

Results Mainly mild adverse events (grade 1-2) occurred during the short-term follow-up, mostly concerning lower urinary tract symptoms. Nearly all resolved between the first and fourth week post treatment. Quality of life assessment showed deterioration in the urinary domain for both treatment protocols. Functional outcome questionnaire results remained stable over time. The reported post-procedural pain was low with a median VAS of 0.5 one day post-IRE, and the length of hospital stay was short (mean of 3 days). Analysis per treatment protocol showed a significant increase of the sexual function between one and four weeks post treatment (p=0.03) in the extended treatment group.

Conclusions Irreversible electroporation can be performed safely in patients suffering from prostate cancer. The adverse events are mostly temporary. Quality of life assessment shows deterioration in the urinary domain; however, functional outcomes remain stable over time.
INTRODUCTION

Prostate cancer (Pca) was the second leading cause of male cancer death in 2014, representing 27% of the total number of new cancers [1]. Pca is currently often diagnosed at an earlier stage because of prostate-specific antigen (PSA) testing, extended prostate biopsies and improved imaging techniques [2,3]. Most patients are now being diagnosed with low- and intermediate risk organ-confined disease [4]. These stages of Pca commonly stay symptomless and may remain non-mortal [5]. Nevertheless, the fear and uncertainty of suffering from cancer are often a motive to choose active treatment over active surveillance [6,7]. Active treatments as radical prostatectomy or radiation therapy are associated with collateral tissue damage causing morbidity including urinary incontinence (0-20%), bowel problems (22-36%) and erectile dysfunction (19-74%) [8–10]. Therefore, several minimally invasive techniques have been proposed to diminish the collateral damage and to spare the urinary sphincter, rectum and neurovascular bundles. The purpose of these so-called focal therapies (FT) is to reduce side-effects without jeopardising the oncological outcomes [11].

Irreversible electroporation (IRE) is a new ablative technique using high-voltage low energy electric pulses to destroy cells by creating persistent micropores. If the IRE procedure is properly executed, essential structures as the urethra, neurovascular bundles and the rectum may stay unharmed potentially lowering post procedural side-effects [12,13]. In recent years, interest in IRE as an ablation modality has grown and studies on IRE have been increasingly reported in the literature [14]. However, data on safety, pain, adverse events and functional outcomes has not yet been published in literature. This study aims to evaluate the safety and quality of life outcomes of extended and focal irreversible electroporation (IRE) in Pca. This registered (NCT001790451) phase I/II, prospective, two-arm interventional, multicentre study was approved by local Ethics Committees within the European Union. The study protocol (including in- and exclusion criteria) has been described elsewhere in detail [15].

MATERIALS AND METHODS

Study design and conduct

Patients with histopathologically Pca, scheduled for a radical prostatectomy as their primary treatment, were invited to participate in the study. The inclusion criteria
were patients who were indicated to undergo a radical prostatectomy and a life expectancy of more than 10 years without prostate calcifications greater than 5 mm. All patients underwent an electrocardiogram evaluation to rule out cardiac rhythm disorders. Informed consent was obtained from all patients after detailed explanation and carefully discussing the potential risks of the trial. Recruitment took place during 2013 and 2014 in two university hospitals (Academic Medical Center, Amsterdam and Sismanoglio general hospital, Athens). The institutional review boards of the two participating institutions in The Netherlands and Greece approved the study.

The primary objective was to determine if the IRE ablation procedure is safe as measured by the total number of device-related, periprocedural- and postprocedural adverse events. The treatment-related toxicity was graded by the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4. CTCAE is widely accepted throughout the oncology research community as the standard grading scale for adverse events (AE). The grade refers to the severity of the AE. All complications were recorded prospectively by the participating centers. A serious adverse event (SAE) was defined as any untoward medical occurrence that requires inpatient hospitalization or prolongation of it, results in persistent or significant disability/incapacity, is life threatening, or results in death.

The secondary objective was to determine quality of life (QoL) as measured by Expanded Prostate Cancer Index Composite (EPIC) and the IPSS Quality of Life score (IPSS-QoL). The EPIC is a validated comprehensive instrument designed to evaluate function and bother after Pca treatment, assessing patients’ urinary, bowel, sexual and hormonal status [16]. Furthermore, post-procedural pain was scored using the visual analogue scale (VAS), at four hours after the procedure, the morning after the procedure and during the planned follow-up visits. Perioperative outcomes and length of hospital stay were documented. Genitourinary side effects were assessed by the following validated questionnaires: the five-item version of the International Index of Erectile Function (IIEF-5), International Prostate Symptom Score (IPSS) and, if required, time of indwelling catheter. The IPSS is based on answers to seven questions concerning urinary symptoms with one additional question concerning quality of life. The total score can therefore range from 0 to 35 (asymptomatic to very symptomatic). The quality of life due to urinary symptom score ranges from 0 to 6 (delighted to terrible). The IIEF-5 is the shortened and simplified version of the International Index of Erectile Function questionnaire (IIEF) and is a validated, widely used questionnaire with high levels of specificity and sensitivity to erectile dysfunction [17]. The questionnaires were administered during visits before the IRE procedure, one week, and four weeks after the procedure. Furthermore, uroflowmetry was also acquired to determine the quality of voiding.
IRE-treatment

Patients were admitted the day before the IRE procedure and were asked to complete all quality of life, functional and pain-related questionnaires. The IRE treatment was performed using the NanoKnife® (AngioDynamics®, Queensbury, NY, USA). The technique utilizes high-voltage microsecond electrical pulses, applied through 19-gauge monopolar needle electrodes leading to the formation of nanopores in the cellular membrane [18,19]. Due to the increased cell membrane permeability and subsequent in- and efflux of ions, a destabilization of the existing cellular transmembrane potential is caused. The cell loses its homeostatic properties resulting in cell death [20–22]. Patients received prophylactic antibiotics two hours preoperatively and general anaesthesia with propofol and/or sevoflurane, sufentanil was induced. After positioning the patients in extended lithotomy position, a transurethral 18 Ch catheter was inserted. The IRE electrodes were transperineally inserted under ultrasound guidance. Prior to the start of pulsing, full paralysis was induced with a rocuronium bolus to prevent patient motion and associated risks. Neuromuscular monitoring was done with a TOF-watch SX acceleromyograph (MSD BV, Haarlem, The Netherlands) aiming for a train-of-four of zero counts and a post-tetanic count of 1-2 twitches during pulsing. To eliminate the risk of pulse-induced cardiac arrhythmias, an EKG-trigger monitor (Accusync®, Milford, Connecticut, USA) was connected to a five-lead EKG to deliver the pulses synchronized with the refractory period of the heart. Ninety pulses were induced between each electrode pair, with the duration of 90 microseconds per pulse delivering an electric field of 1500 V/cm. The active length exposure was set at 1.5 cm. Voltages were adapted following the first 20 pulses if resulting amps were below 20 or above 40 A. Two treatment scenarios were used to assess potential variation in outcomes. Ablation in one lobe of the prostate using ≤3 IRE electrodes was defined as focal. Ablation using 4-6 IRE needles in one of both lobes was called extended.

Follow-up

During the perioperative period and the 4-weeks between the IRE procedure and the RP, complications were documented per type and scored according to the CTCAE. The patient was discharged if successful voiding without significant residuals. In case of urinary retention, an indwelling catheter was reinserted and removed one week later during planned follow-up at the outpatient clinic. A visit at the outpatient clinic was scheduled one week and four weeks after the procedure and a telephone consultation at two weeks post-IRE.
**Statistical considerations**

The Wilcoxon signed rank test was used to evaluate differences between the nonparametric data of the questionnaire scores between the paired samples, measured at baseline and at each follow-up visit. Box plot graphics were computed to describe the outcomes over the follow-up period. Statistical significance was set at $p < 0.05$ and all tests were performed using IBM SPSS statistics, version 14.8.1.

**RESULTS**

Sixteen patients were included from August 2013 to April 2014 and treated across the two centres, Academic Medical Center Amsterdam (n=12) and Sismanoglio hospital Athens (n=4). Patient characteristics show a mean age of $60 \pm 10$ years; median PSA was 8 ng/mL (IQR = 7-13). Ten patients underwent systematic 12-core transrectal biopsies and six patients were diagnosed following targeted or extended biopsies ranging from 13 to 24 cores. Patient characteristics are shown in Table 1.

**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Age in years (mean ± SD)</td>
<td>60.1 ±9.7</td>
</tr>
<tr>
<td>PSA in ng/mL (median; IQR)</td>
<td>8 (7-13)</td>
</tr>
<tr>
<td>Prostate volume mL (mean ± SD)</td>
<td>39 ±12.7</td>
</tr>
<tr>
<td>Number of cores taken (median; IQR)</td>
<td>12 (12-15)</td>
</tr>
<tr>
<td>Number of positive cores (median; IQR)</td>
<td>3 (1-6)</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
</tr>
<tr>
<td>3+3</td>
<td>n = 8</td>
</tr>
<tr>
<td>3+4</td>
<td>n = 3</td>
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<tr>
<td>4+3</td>
<td>n = 3</td>
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<tr>
<td>4+4</td>
<td>n = 2</td>
</tr>
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</table>

**Periprocedural outcomes**

Table 2 summarizes the periprocedural outcomes. The total OR time (time patients were situated in the OR) was on average 104 minutes with a mean anaesthesia time of 81 minutes. The mean duration of the IRE-treatment was 13 minutes, ranging from 2.5 minutes to 27 minutes, depending on the number of inserted electrodes. The patients were normally discharged one day after the procedure resulting in a mean hospitalization time of 3 days. One patient stayed one extra night because of social reasons.
Table 2. Perioperative outcomes of IRE treatment

<table>
<thead>
<tr>
<th>Details</th>
<th>Mean – Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total OR time (minutes)</td>
<td>104 [65 - 140]</td>
</tr>
<tr>
<td>Total anesthetic time (min)</td>
<td>81 [40 - 97]</td>
</tr>
<tr>
<td>IRE-treatment time (min)</td>
<td>13 [2.5 - 27]</td>
</tr>
<tr>
<td>Total hospitalization time (days)</td>
<td>3 [3-4]</td>
</tr>
<tr>
<td>Number of electrodes used</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>n = 1</td>
</tr>
<tr>
<td>3</td>
<td>n = 6</td>
</tr>
<tr>
<td>4</td>
<td>n = 8</td>
</tr>
<tr>
<td>6</td>
<td>n = 1</td>
</tr>
<tr>
<td>Catheterization time (days) (n = 6)</td>
<td>7 [5-9]</td>
</tr>
</tbody>
</table>

All procedures were uneventful. No cardiac arrhythmias occurred during IRE. However, one patient needed an additional dose of rocuronium during the procedure because of severe muscular contractions. Seven patients underwent a focal ablation and nine patients underwent an extended ablation.

Safety and complications during four weeks follow-up

Adverse events

Mild hematuria was noted in 5 patients during the first week following ablation. In all patients, the hematuria resolved spontaneously after 1 to 27 days. No severe hematuria or clotting was observed and no additional treatment or intervention was needed. Two patients reported mild hematospermia, lasting one and thirty days, respectively. During the first week following the IRE-procedure mild (CTCAE grade 1) urinary complaints including: urgency, frequency, painful micturition, or occasional incontinence were observed in seven patients (44%), although none required the use of pads. The miscellaneous grade 1 events included a small perineal swelling, inguinal lymphadenopathy, temporarily swollen testis without fever, bilateral shin pain and/or pain in the lower abdomen without evidence of a urinary tract infection. In seven patients (44%), the urinary complaints were noted as grade 2, because the urgency or frequency was limiting the activities of daily living or pads were used due to urge incontinence. One patient started pelvic floor training. Six of the 16 patients (37.5%) developed a urinary retention one-day post-operative. An indwelling catheter was placed in five patients for the mean duration of 7 days [range 5-9]. The remaining sixth patient needed self-catheterization for 6 days. After the removal of the indwelling catheters, one patient needed self-catheterization for three additional days. One patient developed a urinary tract infection and was treated with oral antibiotics. Another patient experienced diarrhea including hematochezia lasting for two days post-operatively, likely caused by hemorrhoids with no direct relation...
to the procedure. Furthermore, no rectal toxicity was noted, especially no case of
rectourethral fistula or evidence of rectal injury has been observed. One patient
developed a urinary tract infection, which was complicated by an urosepsis. This led
to hospitalization for 6 days during which he was treated with intravenous antibiotics.
No further complications during this admission were registered. None of the patients
experienced life-threatening consequences nor were urgent interventions under
general anesthesia needed. Table 3 and 4 summarizes the adverse events by grade,
incidence and point in time following IRE.

Table 3. Treatment-related toxicity according to CTCAE v4.0

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated</td>
<td>15/16 (94%)</td>
</tr>
<tr>
<td>2</td>
<td>Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living</td>
<td>8/16 (50%)</td>
</tr>
<tr>
<td>3</td>
<td>Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living</td>
<td>1/16 (6%)</td>
</tr>
<tr>
<td>4</td>
<td>Life-threatening consequences; urgent intervention indicated</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Death related to AE</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Post-procedural adverse events CTCAE grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Haematuria</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td></td>
<td>Painful micturition</td>
<td>n = 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hematospermia</td>
<td>n = 2</td>
<td>n = 1</td>
</tr>
<tr>
<td></td>
<td>Pelvic pain</td>
<td>n = 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urgency complaints</td>
<td>n = 2</td>
<td>n = 2</td>
</tr>
<tr>
<td></td>
<td>Frequency complaints</td>
<td>n = 2</td>
<td>n = 2</td>
</tr>
<tr>
<td></td>
<td>Miscellaneous</td>
<td>n = 4</td>
<td>n = 3</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Urine incontinence</td>
<td>n = 3</td>
<td>n = 2</td>
</tr>
<tr>
<td></td>
<td>Urinary retention</td>
<td>n = 6</td>
<td>n = 1</td>
</tr>
<tr>
<td></td>
<td>Urinary tract infection</td>
<td>n = 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urgency complaints</td>
<td>n = 2</td>
<td>n = 1</td>
</tr>
<tr>
<td></td>
<td>Frequency complaints</td>
<td>n = 2</td>
<td>n = 1</td>
</tr>
<tr>
<td></td>
<td>Miscellaneous</td>
<td>n = 1</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>Urosepsis</td>
<td>n = 1</td>
<td></td>
</tr>
<tr>
<td>Grade 4 and 5</td>
<td>None</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quality of life (EPIC, VAS, and IPSS QoL)

Significant differences were observed in one domain of the EPIC questionnaire,
measured before the procedure (baseline) and during follow-up: Quality of life
concerning the urinary function decreased significantly (p=0.01), between pre-IRE
and both the follow-up time points. Quality of life concerning sexual function did not significantly decrease during follow-up, however a significant rise was observed between the first and fourth week after treatment. The quality of life concerning hormonal function and bowel habits did not change significantly following IRE. Mean IPSS quality of life score was 2 (mostly satisfied) at baseline, 3 (mixed feelings) one week postoperatively and returned eventually to mostly satisfied at four weeks following the procedure, showing a significant increase between one and four weeks in follow-up ($p=0.02$). The outcomes are demonstrated in Table 5 and in boxplots in Figure 1.

Table 5. P-values of Wilcoxon signed rank-test for all patients (1), extended treatment group (2), focal treatment group (3).

<table>
<thead>
<tr>
<th></th>
<th>1. All Pre-IRE vs 1 week post</th>
<th>Pre-IRE vs 4 weeks post</th>
<th>1 week vs 4 weeks post</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPIC uro</td>
<td>0.01*</td>
<td>0.01*</td>
<td>0.86</td>
</tr>
<tr>
<td>EPIC bowel</td>
<td>0.72</td>
<td>0.57</td>
<td>0.43</td>
</tr>
<tr>
<td>EPIC sex</td>
<td>0.54</td>
<td>0.17</td>
<td>0.03*</td>
</tr>
<tr>
<td>EPIC hor</td>
<td>0.31</td>
<td>0.65</td>
<td>0.39</td>
</tr>
<tr>
<td>IPSS QoL</td>
<td>0.14</td>
<td>0.19</td>
<td>0.02*</td>
</tr>
<tr>
<td>IPSS</td>
<td>0.37</td>
<td>0.53</td>
<td>0.75</td>
</tr>
<tr>
<td>IIEF</td>
<td>0.71</td>
<td>1.00</td>
<td>0.95</td>
</tr>
<tr>
<td>Qmax</td>
<td>0.11</td>
<td>0.05</td>
<td>0.28</td>
</tr>
<tr>
<td>Residu</td>
<td>0.11</td>
<td>0.13</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>2. Extended Pre-IRE vs 1 week post</td>
<td>Pre-IRE vs 4 weeks post</td>
<td>1 week vs 4 weeks post</td>
</tr>
<tr>
<td>EPIC uro</td>
<td>0.02*</td>
<td>0.04*</td>
<td>0.37</td>
</tr>
<tr>
<td>EPIC bowel</td>
<td>0.87</td>
<td>0.83</td>
<td>0.67</td>
</tr>
<tr>
<td>EPIC sex</td>
<td>0.17</td>
<td>0.44</td>
<td>0.03*</td>
</tr>
<tr>
<td>EPIC hor</td>
<td>0.25</td>
<td>0.21</td>
<td>0.71</td>
</tr>
<tr>
<td>IPSS QoL</td>
<td>0.26</td>
<td>0.30</td>
<td>0.08</td>
</tr>
<tr>
<td>IPSS</td>
<td>0.55</td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td>IIEF</td>
<td>0.80</td>
<td>0.61</td>
<td>0.28</td>
</tr>
<tr>
<td>Qmax</td>
<td>0.67</td>
<td>0.21</td>
<td>0.67</td>
</tr>
<tr>
<td>Residu</td>
<td>0.07</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>3. Focal Pre-IRE vs 1 week post</td>
<td>Pre-IRE vs 4 weeks post</td>
<td>1 week vs 4 weeks post</td>
</tr>
<tr>
<td>EPIC uro</td>
<td>0.17</td>
<td>0.08</td>
<td>0.46</td>
</tr>
<tr>
<td>EPIC bowel</td>
<td>0.65</td>
<td>0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>EPIC sex</td>
<td>0.75</td>
<td>0.35</td>
<td>0.79</td>
</tr>
<tr>
<td>EPIC hor</td>
<td>0.58</td>
<td>0.47</td>
<td>0.46</td>
</tr>
<tr>
<td>IPSS QoL</td>
<td>0.16</td>
<td>0.40</td>
<td>0.10</td>
</tr>
<tr>
<td>IPSS</td>
<td>0.50</td>
<td>0.90</td>
<td>0.60</td>
</tr>
<tr>
<td>IIEF</td>
<td>0.89</td>
<td>0.34</td>
<td>0.20</td>
</tr>
<tr>
<td>Qmax</td>
<td>0.05</td>
<td>0.01*</td>
<td>0.10</td>
</tr>
<tr>
<td>Residu</td>
<td>0.70</td>
<td>1.00</td>
<td>0.29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Pre-IRE vs day 0</th>
<th>Pre-IRE vs 1 day</th>
<th>Pre-IRE vs 1 week</th>
<th>Pre-IRE vs 4 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS All</td>
<td>0.01*</td>
<td>0.01*</td>
<td>0.06</td>
<td>0.18</td>
</tr>
<tr>
<td>VAS Focal</td>
<td>0.07</td>
<td>0.07</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>VAS Extended</td>
<td>0.07</td>
<td>0.07</td>
<td>0.06</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Prior to the procedure, none of the patients experienced any pain relating to their Pca. Four hours after the procedure, the median VAS-score was 1.5 with an interquartile range of 0 to 4.75. The day after the procedure, the median VAS-score was 0.5. The scores were significantly different compared to the pain assessment at
Quality of life and safety outcomes following IRE treatment

Baseline, both with \( p \)-values of 0.01. At the first follow-up visit after one week, the pain score returned to baseline with a mean pain-score of 0.4, showing no significant differences. Overview of the pain scores is shown in boxplots in Figure 2.

![Figure 2. Pain scores pre-IRE and at different time points post-IRE. Significant different outcomes are identified with a star.](image)

Quality of life per treatment protocol

When analyzing the outcomes per treatment protocol (focal versus extended), decreases in the urinary domain of the EPIC with a significant \( p \)-value were observed in the extended ablation group between baseline and one and four weeks follow-up (\( p=0.02 \) and \( p=0.04 \) resp.). In the focal ablation group, no significant differences were noted. In the sexual domain, a significant increase between one and four weeks post-treatment was only observed (\( p=0.03 \)) in the extended treatment group. Urinary and Erectile function (IPSS + IIEF-5 + uroflowmetry) IPSS outcomes did not differ significantly between baseline, 1 week and 4 weeks postoperatively. Mean values were 11, 12 and 12, respectively. The erectile function determined by the IIEF-5 demonstrated no significant difference between baseline and follow-up. Furthermore, uroflowmetry showed a mean maximal flow of 17.2 mL/sec at baseline, followed by 14.1 mL/sec at one week and 14.3mL/sec at four weeks. These values, as well as the measured residuals after voiding, were not statistically different between the respective observations. The data are demonstrated in boxplots in Figure 3.

Urinary and erectile function per treatment protocol

No differences were seen between the two treatment groups concerning the urinary or erectile functions except for the maximum flow of the focal ablation group. The flow (Qmax) of this patient group decreased significantly four weeks post-treatment compared to baseline (\( p=0.01 \)), where it did not in the extended group. The data are shown figure 3.
Chapter 7

Figure 3. IPSS, IIEF-5, uroflowmetry (Qmax) and residuals after voiding assessed at baseline, one and four weeks after IRE procedure. Significant differences are identified with a star.

DISCUSSION

The present study on safety and quality of life shows that ablations using IRE, either focally or extended executed can be performed safely. During the four weeks follow-up, mainly grade 1 and 2 AE occurred, mostly concerning lower urinary tract symptoms. Nearly all resolved between the first and fourth week post-IRE. One grade 3 AE (urosepsis) required a readmission whereupon the patient recovered rapidly. This urosepsis was possibly induced by the required indwelling catheter and advanced
age (70y) of the patient. However, urosepsis is a serious complication and the possible occurrence of urosepsis should be taken into account during IRE follow-up. The quality of life, urinary and erectile function outcomes stay stable over time during the short follow-up. The high pain scores, reported directly after the procedure, could be influenced by indwelling catheters, which occasionally lead to bladder contractions. The decrease of the quality of life measured by the EPIC (urinary domain) was significant in the extended ablation group and not in the focal group, suggesting that ablation using four or more electrodes may cause more urinary complaints than using fewer electrodes. In the focal ablation group, the maximal flow decreased significantly, but in the extended group more patients needed an indwelling catheter during the first week after treatment. This could have been supportive in blocking the swelling of the prostate post-treatment.

The aim of focal therapy is to offer Pca patients reliable oncological control whilst preserving their quality of life. To achieve both cancer control and limited treatment-related morbidity, it is of utmost importance to treat the accurate patient population. Recent years, several multidisciplinary consensus projects have established criteria for selection of candidates for focal therapy in prostate cancer [23–26]. Furthermore, it is essential to select the most appropriate ablative energy source [27]. The various options for minimally invasive tissue ablation have different technical characteristics that can be advantageous or disadvantageous depending on the individual patient’s clinical situation. The two most frequently used techniques are high-intensity focused ultrasound (HIFU) and cryotherapy. An overview for these techniques of post-treatment complications, functional outcomes and quality of life assessment is shown in Table 6. However, implementation of these treatments in clinical practice has not been preceded by a proper safety study. New surgical innovations require structured evaluation, described in the recommendations of the IDEAL collaboration [29]. The phase I-II study is limited by the small cohort size and short follow-up period. But in this period, the results seem encouraging. Though, a successive powered (randomized) study is necessary to confirm these outcomes. Another limitation is that some questionnaires were filled in shortly before the hospital admission for the IRE procedure, which could have led to emotional bias. However, we perceive the results to be promising in comparison with the results of the more mature ablation therapies as HIFU and cryoablation. Research into conventional focal ablative therapies in the prostate is very heterogeneous, due to differences in trial protocols, patient number and duration of follow-up. Table 6 provides a structured overview of complications, quality of life and functional outcomes of recent HIFU and cryotherapy trials in prostate cancer. Several studies report serious complications as rectourethral fistulas and urethral strictures [36,42-43,46,48-50,52].
<table>
<thead>
<tr>
<th>FT</th>
<th>Complications</th>
<th>Urinary continence</th>
<th>Erectile function</th>
<th>Rectal toxicity</th>
<th>Quality of life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beerlage et al. [32]</td>
<td>HIFU</td>
<td>NR</td>
<td>NR</td>
<td>Rectourethral fistula 0/14 (0%) Perineal pain 14/14 (100%)</td>
<td>NR</td>
</tr>
<tr>
<td>Bahn et al. [33]</td>
<td>Cryo</td>
<td>NR</td>
<td>28/28 (100%)</td>
<td>24/27 (88.8%)</td>
<td>NR</td>
</tr>
<tr>
<td>Onik et al. [34]</td>
<td>Cryo</td>
<td>NR</td>
<td>24/25 (96%)</td>
<td>44/51 (85%)</td>
<td>NR</td>
</tr>
<tr>
<td>Ellis et al. [35]</td>
<td>Cryo</td>
<td>NR</td>
<td>54/55 (96.4%)</td>
<td>24/34 (70.6%)</td>
<td>Rectourethral fistula 0/34 (0%)</td>
</tr>
<tr>
<td>Muto et al. [36]</td>
<td>HIFU</td>
<td>Urethral stricture 1/25 (4%)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Robinson et al. [37]</td>
<td>Cryo</td>
<td>NR</td>
<td>NR</td>
<td>27/122 (22%)</td>
<td>NR</td>
</tr>
</tbody>
</table>

Truesdale et al. [38] | Cryo | NR | 77/77 (100%) | NR | NR |

Li et al. [39] | HIFU and cryo | NR | NR | Cryo: 22/47 (46.8%) | HIFU: 36/55 (65.5%) |

El Fegoun et al. [40] | HIFU | Urinary retention 1/12 (8%) | Urinary tract infection 2/12 (16%) | 12/12 (100%) | NR |

Donnelly et al. [41] | Cryo | Geritourinary: - Urgency/frequency 76/117 (64%) - Retention 26/117 (22%) - Pain 3/117 (2.6%) - Diarrhea 8/117 (6.8%) - Fecal incontinence 9/117 (7.7%) - Proctitis 2/117 (1.7%) - Gastrointestinal pain 17/117 (14.5%) - Gastrointestinal bleeding 9/117 (7.7%) | 79/117 (67.5%) | 14/48 (29.1%) | NR |

Ahmed et al. [42] | HIFU | Urinary stricture 1/20 (5%) | 18/20 (90%) | 19/20 (95%) | - | No. significant difference |

Ward et al. [43] | Cryo | Urinary retention 6/518 (1.1%) | 499/507 (98.4%) | 169/291 (58.1%) | Rectourethral fistula 1/507 (0.1%) | NR |

Temporary deterioration of urinary function and bowel function. Permanent deterioration of sexual function.
<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Complications</th>
<th>Quality of life and safety outcomes following IRE treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahn et al. [44]</td>
<td>Cryo NR</td>
<td>7/42 (16.7%)</td>
<td>36/42 (86%) Rectourethral fistula 0/73 (0%) NR</td>
</tr>
<tr>
<td>Ahmed et al. [45]</td>
<td>HIFU</td>
<td>Urinary retention 1/41 (2.4%)</td>
<td>100% SR 1/41 (2.4%) Significant deterioration</td>
</tr>
<tr>
<td>Barret et al. [46]</td>
<td>HIFU cryo</td>
<td>Urinary retention 9/106 (8.5%)</td>
<td>100% NR 9/106 Significant deterioration</td>
</tr>
<tr>
<td>Napoli et al. [47]</td>
<td>HIFU NR</td>
<td>3/5 (60%)</td>
<td>NR</td>
</tr>
<tr>
<td>Crouzet et al. [48]</td>
<td>HIFU</td>
<td>Urinary tract infection 39/1002 (3.9%) Urinary retention 76/1002 (7.6%)</td>
<td>100% No sign. diff. in IIEF Perineal fistula 1/48 (2%) NR</td>
</tr>
<tr>
<td>Durand et al. [49]</td>
<td>Cryo</td>
<td>Urinary retention 7/48 (15%)</td>
<td>100% No sign. diff. in IIEF Perineal fistula 1/48 (2%) NR</td>
</tr>
<tr>
<td>Mendez [50]</td>
<td>Cryo</td>
<td>Urinary retention 7/3%</td>
<td>100% No sign. diff. in IIEF Perineal fistula 1/48 (2%) NR</td>
</tr>
<tr>
<td>Barqawi et al. [51]</td>
<td>Cryo</td>
<td>No severe complications</td>
<td>100% 1.5 point decrease of American Urological Association Symptom score</td>
</tr>
<tr>
<td>Ahmed et al. [52]</td>
<td>HIFU</td>
<td>Dysuria 9/56 (16.1%) Hematuria 36/56 (64.3%) Urinary debris 2/56 (42.9%)</td>
<td>46/50 (92.0%) 30/39 (76.9%) NR Very little impact of health-related quality of life</td>
</tr>
</tbody>
</table>

HIFU: high-intensity focused ultrasound; cryo: cryotherapy; NR: not reported. sign: significant; diff: difference.
Our series did not show any persisting toxicity; however it has to be noted that, at this stage of research, our patient number is considerable lower than in most reported series concerning conventional focal therapies. Our results are in accordance with the initial assessment of safety of IRE treatments performed by Valerio et al. [13]. That study shows the results of 34 patients treated with IRE, where 12 patients had grade 1 and 10 patients had grade 2 complications. No grade 3 adverse events occurred. Functional outcomes, based on physician-reports, showed a preservation of potency of 95% of the men potent before IRE and all men remained continent after treatment at six months post-IRE.

When comparing IRE with results published in a systematic review [11] of several focal techniques, IRE might be beneficial in terms of adverse events and functional outcomes. The overall OR- and procedural time is clearly shorter compared to other FT, which is advantageous in terms of healthcare costs. However, a multicentre randomized controlled trial with larger patient numbers is necessary to confirm the low-morbidity rates. Recently, the Clinical Research Office of the Endourological Society (CROES) has launched a RCT on IRE (comparing two ablation protocols) in which eight European centers will participate to assess both safety and efficacy. Additionally, an international web-based registry database has been designed to address the clinical data associated with IRE in prostate cancers [30].

Since focal treatment is still considered investigational and out-of-field disease following FT may occur, it is essential that any focal therapy modality does not compromise the efficacy of salvage therapy, if needed. In all cases, surgery was feasible and the experienced surgeons who performed the RPs did not notice abnormalities or difficulties. No rectal injuries or rectal fistulae were observed during the surgery itself and postoperatively. However, it is known from the literature that salvage radical prostatectomy for radiation-recurrent disease causes significant deterioration of quality of life in terms of sexual and urinary dysfunction [31]. Although the functional outcomes following the post-IRE radical prostatectomy were beyond the scope of this study, it is very well possible that patients may experience more surgical-related morbidity due to the prior IRE.

**CONCLUSIONS**

IRE ablations can be performed safely in patients suffering from prostate cancer. The adverse events are mostly temporary and mainly grade 1 and 2 side effects are observed. Quality of life assessment shows deterioration in the urinary domain in both treatment protocols; however functional outcomes remain stable over time.
REFERENCES


Chapter 8

Histopathological outcomes after irreversible electroporation in prostate cancer: Results of an ablate-and-resect study

W van den Bos, RR Jurhill, DM de Bruin, CD Savci-Heijink, AW Postema, PGK Wagstaff, BG Muller, IM Varkarakis, A Skolarikos, PJ Zondervan, MP Laguna Pes, TM de Reijke, JJMCH de la Rosette

Journal of Urology – 2016 March 19 [Epub ahead of print]
ABSTRACT

Purpose Irreversible electroporation (IRE) is a tissue ablation modality that uses high-voltage electric energy to induce an increase of cell membrane permeability. This causes a destabilization of the existing cellular transmembrane potential leading to cell death, due to the inability to maintain cellular homeostasis. This phase I-II study was designed to evaluate the histopathological outcomes of IRE to prostate and surrounding tissue in radical prostatectomy (RP) specimens.

Materials and methods Sixteen prostate cancer patients underwent an IRE ablation without curative intent, followed by a RP scheduled four weeks later. For histopathological examination of the prostate, whole-mounted tissue slices were examined by dedicated genitourinary pathologists. The borders of the ablation zone and residual tumour were outlined on the slides.

Results The IRE ablation zones were characterized as areas of fibrosis, necrosis and loss of epithelial tissue in terms of denudation in the glandular structures. The ablation zone was well demarcated, showing trenchant delineations between the viable and non-viable tissue. The ablated tissue showed mild to moderate inflammation, with atrophic cells in one case. The area was surrounded by haemorrhage at the location of the electrodes. No skip lesions, no viable tissue, were seen within the ablation zone. Fibrinoid necrosis of the neurovascular bundle was observed in 13 patients and denudation of the urothelium of the prostatic urethra in 9 patients.

Conclusions Histopathological assessment of the prostate four weeks after IRE ablation showed sharp-demarcated fibrotic and necrotic tissue within the ablation zone. No viable tissue was observed within the IRE ablation zone.
INTRODUCTION

Because of the intensified use of prostate-specific antigen (PSA), the incidence of prostate cancer (PCa) has increased, and it is more often diagnosed at an earlier age and earlier stage [1,2,3]. In spite of the fact that patients with low-risk prostate cancers are nowadays counselled for active surveillance, a number of these patients still requests active treatment including surgery and radiation therapy, which may cause a significant burden. For this reason, the urological community is searching for an approach that is less harmful but still safeguards excellent oncological outcomes. Therefore, a variety of minimally invasive techniques have been evaluated for the ablation of prostate cancer. The aim of this approach, named focal therapy, is to target and treat only the clinically significant identified cancer areas whilst leaving the benign tissue and adjacent structures untouched hence minimizing the treatments’ morbidity and reducing the impact on quality of life. A novel technique used for focal ablation is irreversible electroporation (IRE). This tissue-ablation modality uses high voltage electric energy inducing an increase of cell membrane permeability and subsequent in- and efflux of ions [4–6]. A destabilization of the cellular transmembrane potential, together with secondary thermal effects, renders the cell unable to maintain its homeostatic properties, resulting in cell death [7–10]. The present phase I-II study was designed to evaluate the histopathological outcomes of IRE to prostate and surrounding tissue in radical prostatectomy specimens.

MATERIALS AND METHODS

Population

Patients with biopsy-proven prostate cancer were prospectively enrolled to undergo an IRE procedure four weeks prior to the scheduled radical prostatectomy (RP). The patients were diagnosed by transrectal ultrasound (TRUS) guided-prostatic needle biopsy, indicated by an increased PSA concentration or abnormal digital rectal examination. The study was conducted in two academic centres: AMC University hospital in Amsterdam, The Netherlands and Sismanoglio hospital in Athens, Greece. Both centres received approval from the Institutional review board and informed consent was obtained from all patients. The study protocol is registered in the database of clinicaltrials.gov (NCT01790451) and published by van den Bos et al. [11].
**Procedure**

IRE treatment was performed using monopolar needle electrodes (Nanoknife, AngioDynamics, Latham, New York) which were placed in the prostate transperineally with active length of the electrodes of 1.5 cm, using interelectrode distances of 1-2 cm. The electrical parameters consisted of: 90 pulses of 90 µsec, at a voltage of 1200 to 2100 V/cm, resulting in a current of 15-40 A. The IRE procedure specifications are listed in Table 1. The numbers of the electrodes as well as the needle-configurations were altered per patient in order to evaluate the various ablation effects. The placement of the electrodes and the procedures were performed under transrectal ultrasound guidance. The electrodes were positioned to target the major extent of the tumour, but the ablation was executed without curative intent. Exact electrode configurations were documented for each patient. Patients were treated under general anaesthesia with muscle paralysis to decrease body muscle spasms induced by the high voltage. The IRE was administered with continuous ECG-synchronization to prevent pulse-induced cardiac arrhythmias. PSA-levels were determined at inclusion, the day after the IRE-procedure and at the day of admission before the RP. The statistical significance of the differences in PSA-levels between the time points was examined using the non-parametric Wilcoxon signed ranks test (SPSS, version 20). A significance level of $p<0.05$ was considered statistically significant.

**Table 1. IRE procedure specifications**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Number of electrodes</th>
<th>Voltage-to-distance ratio (V/cm)</th>
<th>Amperage (A)</th>
</tr>
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</tr>
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<td>4</td>
<td>1500</td>
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</tr>
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<td>Pt. 4</td>
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<td>3</td>
<td>1500-1800</td>
<td>26-35</td>
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<td>Pt. 6</td>
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<td>1350-1500</td>
<td>25-45</td>
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<td>3</td>
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<td>22-28</td>
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<td>1500-1800</td>
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<td>21-31</td>
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<td>1500-1800</td>
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<td>Pt. 15</td>
<td>3</td>
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</tr>
<tr>
<td>Pt. 16</td>
<td>3</td>
<td>1500</td>
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</tbody>
</table>
Radical prostatectomy
Approximately four weeks were scheduled between IRE treatment and RP for all patients. The RP has been performed using laparoscopic (12 patients) and open (4 patients) techniques. The progress of the surgical procedure and any unusual findings encountered during the surgery were reported by the three urological surgeons (PL/IV/PZ), surgeons with more than 20, 10 and 10 years of experience performing this surgery.

Histopathological examination
The histological examination of the prostate specimen from both participating centres was performed at the department of pathology in the AMC, Amsterdam by two pathologists (CDS-H and RJ), with 15 and 6 years of experience, respectively. Following surgical excision, inspection of the prostate was performed including size measurements and weighing. The prostate was then inked with different colours in order to preserve the orientation of the planes (Figure 1). Part of an indwelling catheter was placed in the prostatic urethra in order to preserve the anatomy during the fixation. The specimen was fixed in formalin for a period of 1 week and sliced perpendicularly to the rectum. In the AMC, whole-mounted tissue slices from apex to base of approximately 4 mm thickness were obtained (Figure 2). In the Sismanoglio hospital, the slices were cut into two to four parts and mapped in a routine scheme. The sections were mounted on slides and stained with haematoxylin-eosin. Both the ablated and non-ablated areas were examined carefully by light microscopy. Immunohistochemical stains (p63/AMACR and 34BE12) were used if deemed necessary by the pathologists. The borders of the ablation zone and residual tumorous tissue were outlined on the slides. Detailed analysis was performed to rule out skip lesions, defined as viable cells completely enclosed by ablated tissue. The ablated and non-ablated areas were examined and relevant histopathology findings were scored accordingly. Presence of viable tumour cells and viable non-tumoral cells were estimated as a percentage of the ablated and non-ablated zones separately.
Figure 1. Inked prostate specimen with ruler (in centimeters).

Figure 2. Whole-mounted tissue slices from apex to base of approximately 4 mm thickness.
RESULTS

Patient characteristics
The patient characteristics were on average as follows: age 60 years (range 44-75; SD 9.7); prostate volume on ultrasound 39 mL (range 19-60; SD 12.7), median serum PSA of 6.9 ng/mL (IQR 5.4-12.28). The biopsies showed in 8 patients Gleason score 3+3, in 3 patients Gleason score 3+4, in 3 patients Gleason score 4+3, and in two patients 4+4, distributed in unilateral disease in 11 patients and bilateral disease in 5 patients. Patient and cancer characteristics are presented in Table 2.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Prostate volume (mL)</th>
<th>Prostate biopsy results</th>
<th>Tumour distribution</th>
</tr>
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<td>Unilateral</td>
</tr>
<tr>
<td>Pt. 2</td>
<td>30</td>
<td>4 + 3</td>
<td>Bilateral</td>
</tr>
<tr>
<td>Pt. 3</td>
<td>57</td>
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<td>Unilateral</td>
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<td>Pt. 4</td>
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<td>Unilateral</td>
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<td>4 + 4</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Pt. 6</td>
<td>42</td>
<td>3 + 3</td>
<td>Unilateral</td>
</tr>
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<td>21</td>
<td>3 + 3</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Pt. 8</td>
<td>40</td>
<td>3 + 3</td>
<td>Bilateral</td>
</tr>
<tr>
<td>Pt. 9</td>
<td>50</td>
<td>3 + 3</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Pt. 10</td>
<td>37</td>
<td>4 + 3</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Pt. 11</td>
<td>25</td>
<td>3 + 4</td>
<td>Bilateral</td>
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<tr>
<td>Pt. 12</td>
<td>50</td>
<td>3 + 4</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Pt. 13</td>
<td>28</td>
<td>3 + 4</td>
<td>Unilateral</td>
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<tr>
<td>Pt. 14</td>
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<td>3 + 3</td>
<td>Unilateral</td>
</tr>
<tr>
<td>Pt. 15</td>
<td>31</td>
<td>3 + 3</td>
<td>Bilateral</td>
</tr>
<tr>
<td>Pt. 16</td>
<td>48</td>
<td>4 + 4</td>
<td>Bilateral</td>
</tr>
</tbody>
</table>

The median number of positive cores was 3 (IQR 1-6). PSA levels increased significantly at day one post-IRE with a median of 78.6 ng/mL (IQR 33.6-104.1) (p-value: 0.001). Four weeks after the procedure, the levels lowered to a median of 4.6 ng/mL (IQR 2.8-8.4) (Figure 3). These levels did not significantly differ from the PSA measured before the procedure (p-value: 0.061).
Macroscopic examination of the radical prostatectomy specimen

Macroscopic examination showed an ellipsoid-shaped haemorrhagic area surrounding small pale discoloured zones at the centre of the IRE ablation zone (Figure 2). The well-demarcated ablation volumes ranged from 5 to 40% of the prostate and extended from the capsule to the prostatic urethra. One prostate specimen did not show any macroscopically assessable ablation zone. Tracts of the electrodes could not be identified.

Microscopic examination of the radical prostatectomy specimen

Microscopic assessment of the ablation zone showed areas of fibrosis, necrosis and ghost-tubuli with eosinophilic cytoplasm in 15 out of 16 patients. This area was surrounded by a hemorrhagic area corresponding with the location of the electrodes on ultrasound (Figure 4). The ablation zone was well demarcated, showing a trenchant delineation between the viable and non-viable tissue (Figure 5). One prostate specimen showed only fibrosis without a necrotic component. The ablated tissue showed mild to moderate inflammation in all cases with a focal severe component with atrophic cells in one case. Glandular hyperplasia was present in 11 prostates. Additionally, basal cell hyperplasia and transitional cell metaplasia were identified in 10 and 1 cases, respectively. No skip lesions (no viable tissue) were seen in the ablated area. The prostate capsule was affected by the IRE treatment in 12 of 16 cases, showing invasion of adipocytes and lipophages in the capsule.
IRE effects were observed extending in the neurovascular bundle of 13 patients at the side and in the zone of ablated area. The nerve bundles outside the boundaries of the ablation zone were mainly without damage. In 12 of the 13 unilateral IRE ablations, no damage was observed to the contralateral neurovascular bundle. In the
remaining unilateral ablation, the histopathology report concerning the contralateral neurovascular bundle was inconclusive. Nerve damage was recognized as eosinophilic degeneration of the cytoplasm and pyknotic nuclei of the nerves, without any sign of regeneration (Figure 6). The prostatic urethra was affected in 9 patients showing denudation of the urothelium (Figure 7). Examination of the tissue outside the ablation zone showed multifocal tumorous tissue in 15 patients. The diameter of the dominant tumour area ranged from 3 to 18 mm and tumour volume did not exceed 10% in 13/16 cases and three cases showed tumour invasion in maximally 50% of the tissue. Four tumors extended into the extraprostatic tissue. The tumors in the RP specimens varied in Gleason scores ranging from 3+3 to 3+5, with 15 specimens having a Gleason score of either 6 or 7 and only one case having a Gleason score of 8. R0-resection was reached in thirteen cases meaning complete removal of all tumors after microscopic examination of margins showing no tumour cells. R1-resection was reached in the three remaining cases. The tumours were staged as follows: 10 patients pT2cR0, 1 pT2cR1, 3 patients pT3a R0, 1 patient pT3aR1 and it was presumed that IRE had been curative in the one prostate where no remaining carcinoma was found. An overview of the patient-specific histopathology results is provided in Table 3.

Figure 6. Affected neural tissue in neurovascular bundle.
Figure 7. Prostatic urethra with denudation of urothelium.

Table 3. Histopathology results

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Glandular hyperplasia</th>
<th>Atrophy</th>
<th>Inflammation</th>
<th>Fibrosis</th>
<th>Necrosis</th>
<th>Gleason score</th>
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<td>Yes</td>
<td>Yes</td>
<td>Moderate/Severe</td>
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<td>Yes</td>
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<td>Bilateral</td>
<td>pT2cNxMX</td>
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<td>No</td>
<td>Moderate</td>
<td>Yes</td>
<td>Yes</td>
<td>3 + 4</td>
<td>Bilateral</td>
<td>pT3aNxMx</td>
</tr>
<tr>
<td>Pt. 4</td>
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<td>Mild</td>
<td>Yes</td>
<td>Yes</td>
<td>3 + 3</td>
<td>Bilateral</td>
<td>pT3aNxMx</td>
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<tr>
<td>Pt. 5</td>
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<td>No</td>
<td>Mild</td>
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<td>-</td>
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<td>Pt. 8</td>
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<td>Yes</td>
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DISCUSSION

This is the first comprehensive study reporting on histopathological changes of prostate cancer tissue following IRE ablation. Analysis of the ablation zone demonstrated sharply demarcated necrotic and fibrotic prostate tissue, with the exception of one patient. The patient was treated using two electrodes inserted in the right lobe; however the specimen showed bilateral fibrosis, without the necrotic component, in a confluent pattern spread in both lobes. During the ablation, the IRE generator reported a current rise of 3 amps (from 15 to 18 amps), which is considered low by Dunki-Jacobs et al. [12] The purpose of their study was to determine if tissue resistance can be used to predict successful tumour ablation in pancreatic cancers. The authors stated that a change of approximately 12 to 15 amps is needed for successful tumour ablation. Furthermore, the fibrosis was present throughout the prostate as a whole, leading us to presume that this was pre-existent as a result of age and chronic inflammation.

IRE effects extended in the neurovascular bundle of 13 patients at the side of the ablated lobe. However, the nerve bundles outside the ablation zone were without damage. In all conclusively examined unilateral IRE ablations (12), no damage was observed to the contralateral neurovascular bundle. During the short-term follow-up of 4 weeks, quality of life outcomes concerning sexual function showed no significant decrease. The erectile function determined by the International Index Erectile Function-5 demonstrated no significant difference as well. The prostatic urethra was affected in 9 patients and the quality of life concerning the urinary function decreased significantly post-IRE [13].

Analysis of imaging studies performed four weeks after the ablations showed that ablation volumes on multiparametric-MRI (mpMRI) and contrast-enhanced ultrasound (CEUS) correspond with the ablation volumes on PA [14]. It demonstrates the feasibility of imaging follow-up using mpMRI and CEUS. Both CEUS and contrast-enhanced MRI are based on tissue perfusion and both modalities clearly show a loss of enhancement that represents devascularisation and thus tissue destruction (Figure 8). Analysis of the targeted area within the electrode configuration and the achieved ablation zone showed that the latter one is ~2.7 times larger than the electrode configuration [15].
Valerio et al. performed the first clinical study using IRE as primary treatment for prostate cancer [16]. The study reports oncological outcomes of 24 patients having a follow-up of at least 6 months showing suspicious residual disease on multiparametric MRI in six patients. Two patients remained on active surveillance and three had an additional focal ablation using IRE or HIFU. One underwent transperineal targeted biopsy confirming a residual Gleason 3+4 tumour and underwent a radical prostatectomy. Ting et al. performed IRE ablation in 25 prostate cancer patients and 21 men underwent transperineal template-guided mapping biopsy (TTMB) at 7 months post-IRE [17]. In these patients, the ablation zone (in-field) was histologically free of tumor cells on TTMB. In 16 patients, no significant disease was found and 8 patients were free of cancer on histopathology assessed during follow-up. Significant cancer was detected on biopsy of the tissue adjacent to the treated area in four patients. Biopsies performed outside the ablation zone showed significant disease in one patient. Overall, 5 of the 21 patients (24%) who underwent follow-up biopsies had proven significant cancer. Three of the patients will remain on active surveillance, one will undergo a second IRE-procedure and one is treated by a salvage robot-assisted laparoscopic prostatectomy. No analysis of the specimen was reported.
Our study has limitations. First, analysis of the histopathology beyond the short-term follow-up of four weeks was not possible because postponing the surgery was ethically not feasible. Therefore, the long-term effects of the damaged nerves in the NVB and the recovery of the urethra could not be assessed. A second limitation is the small sample size of included patients. The number was chosen in accordance with the ethical committee of both participating hospitals, and sixteen patients was the maximum in this phase of research. However, the meticulous analysis of the histopathology specimens has yielded vital results on prostate IRE feasibility and safety, allowing for a safe and scientifically valid transition to IRE follow-up studies in prostate cancer. A multicentre randomized controlled trial on IRE has launched by the Clinical Research Office of the Endourological Society (CROES). Two ablation protocols are compared assessing on safety and efficacy. Finally, an international prostate IRE registry has been designed to centrally collect clinical data of IRE in prostate cancers [18].

CONCLUSIONS

Histopathological assessment of the prostate four weeks after ablation using irreversible electroporation showed sharp-demarcated fibrotic and necrotic tissue within the ablation zone. No viable cells were detected within the ablation zone. Deterioration to structures as urethra, prostatic capsule and neurovascular bundles may occur, though the long-term effects remain unknown.
REFERENCES

Chapter 9

What is still needed to make focal therapy an accepted segment of standard therapy?

W van den Bos, BG Muller, B Ehdaie, P Scardino, JMJCH de la Rosette

Published in Current Opinion in Urology 2014,24:247–255
ABSTRACT

Purpose of review Focal therapy is gaining interest and this organ-preserving treatment is heading towards becoming an alternative for the conventional surgery and radiation. The purpose of this review is to determine what evidence is required to make focal therapy a viable option for treatment of localized prostate cancer.

Recent findings There is still a lack of high-level evidence for the different focal treatment methods. The early stage focal therapy trials are conducted including a various selection of patients and different pretreatment assessment and follow-up, resulting in incomparable data. Recent literature shows it is paramount to extend the amount of biopsies and to alter the way of taking the biopsies with the template-assisted or image-guided approach. To date, multiparametric MRI is the most effective imaging technique in selecting patients for focal therapy.

Summary Focal therapy trials are at the early stage of clinical development, with the majority still being phase I studies. To make focal therapy an accepted segment of standard therapy, it needs to proceed towards phase II and III trials. These trials should be conducted with an effective trial design, what will lead to more comparable oncological, functional and quality of life outcomes. Furthermore, it is essential to improve the localization of tumor foci in order to increase the accurateness of spatial targeting of cancer.
INTRODUCTION

Prostate cancer is the most prevalent form of cancer in men in Western countries. Currently, due to a lack of reliable techniques for prostate cancer imaging, treatment options are often restricted to radical treatments, which carry significant risks of permanent side effects, such as incontinence or impotence and bowel issues. Therefore focal therapy in prostates has been proposed as an alternative. Magin et al. started in 1980 with thermal destruction of the canine prostate by high intensity microwaves [1]. Since then, a variety of focal ablation techniques have been introduced to the minimal invasive treatment of prostate cancer. These techniques include focal brachytherapy, cryoablation, high-intensity focused ultrasound (HIFU), laser ablation therapy, radiofrequency ablation, photodynamic therapy (PDT) and irreversible electroporation (IRE). The different ablation mechanisms are explained in Figure 1. In all of the aforementioned therapies, there is a scarcity of long-term data on the efficacy and on metastasis-free, prostate cancer-specific or overall survival. The quality of the evidence is low to moderate, with no study yielding a level of evidence >2b [2]. However, whole gland cryosurgery has been accepted as a true therapeutic alternative by the guidelines of the American Urological Association (AUA), despite of this lack of high-level evidence from prospective randomized trials to support the role of cryosurgery over the other therapeutic options [3]. This is caused by the acceptable health-related quality of life (HRQL)-outcomes and low costs compared to alternative local treatment options and due to the short-term PSA relapse-free survival outcomes after whole-gland cryoablation [4]. HIFU is considered an experimental treatment by the AUA and has potentially the same therapeutic efficacy as established surgical and non-surgical options, as the additional advantage of reduced therapy-associated morbidity [5]. No any other focal treatment is considered as therapeutic or experimental alternative so the major unanswered question remains: What is still needed to make focal therapy an accepted segment of standard therapy?
Figure 1. Ablation mechanisms of laser ablation therapy, cryotherapy, high-intensity focused ultrasound, radiofrequency ablation, irreversible electroporation, brachytherapy and photodynamic therapy.
LITERATURE SEARCH

Studies were identified by electronic search of Medline (through Pubmed) with prespecified English language and human-studies restrictions. Literature from clinical trials and reviews with a full-text availability were included. In addition, registered trials were retrieved from trials registries (ClinicalTrials.gov and the International Standard Randomised Controlled Trial Number). We conducted a search of ongoing trials to allow us to determine the current thinking on patient selection, tumour localization, imaging and types of outcome measures and follow-up that investigators in this area are now using. The search strategy was as follows: ‘focal treatment’ OR ‘focal therapy’ OR ‘tissue-preserving’ OR ‘tissue-preservation’ OR ‘subtotal’ OR ‘cryosurgery’ OR ‘cryotherapy’ OR ‘cryoablation’ OR ‘high-intensity focused ultrasound ablation’ OR ‘HIFU’ OR ‘photodynamic therapy’ OR ‘PDT’ OR ‘laser therapy’ OR ‘laser ablation’ OR ‘radiofrequency ablation’ ‘focal brachytherapy’ OR ‘irreversible electroporation’ OR ‘IRE’ AND ‘carcinoma’ OR ‘cancer’ OR ‘neoplasm’ OR ‘neoplasms’ OR ‘neoplasm’ OR ‘neoplasma’ OR ‘neoplasms’ OR ‘malignancy’ OR ‘malignant’ OR ‘adenocarcinoma’ OR ‘adenocarcinom’ OR ‘tumor’ OR ‘tumour’ OR ‘cancers’ AND ‘prostate’ OR ‘prostatic’ OR ‘prostatae’. (Figure 2)

Figure 2. Flowchart of literature search
PATIENT SELECTION

Clinical trials involving new interventional treatments are usually classified into four phases (Figure 3). Most focal therapy trials are still assessing the safety and starting to determine the efficacy (Table 1). To proceed forward from experimental treatments to accepted therapies, high level of evidence is needed. Trial designs with comparable outcomes need to become standardized with respect to eligibility criteria and primary endpoints [18]. Currently, the inclusion criteria of recent, active and recruiting primary treatment clinical trials vary widely (Table 1). Agreement on candidate selection moved recently from low-risk cancer, Gleason 6, clinical stage T2a and estimated life expectancy >10 years to patients with T1c-T2a tumours, Gleason 3 + 3 or 3 + 4 and a life expectancy of > 10 years [19] and [20]. In case of focal brachytherapy, consensus agreement was made to select patients with unilateral disease with lesions size ≤ 0.5 mL, tumour stage ≤ T2b, Gleason 3 + 3 or 3 + 4, PSA ≤ 15, life expectancy > 10 years and prostate size ≤ 60 mL [21].

Figure 3. Phases of clinical research

- **Phase I**
  - Checking for safety
  - Sample size: 10-20 (healthy) volunteers

- **Phase II**
  - Checking for efficacy
  - Sample size: 100-200 patients

- **Phase III**
  - Confirm findings phase II
  - Sample size: 200 – 1000 patients

- **Phase IV**
  - Long term safety surveillance
  - Sample size: large population
Table 1. Overview of published, completed, ongoing and recruiting focal therapy trials

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<th>Year</th>
<th>Technique</th>
<th>Guidance</th>
<th>Stage</th>
<th>Age</th>
<th>PSA</th>
<th>GS</th>
<th>Phase</th>
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<td>Photothermal therapy</td>
<td>3D US &amp; MR fusion</td>
<td>T1c – T2a</td>
<td>-</td>
<td>&lt; 10</td>
<td>≤ 6</td>
<td>I</td>
<td>&lt; 30% cores positive for cancer</td>
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<td>Cryoablation</td>
<td>Ultrasound</td>
<td>T1c – T2b</td>
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<td>-</td>
<td>-</td>
<td>II</td>
<td>-</td>
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<td>Laser therapy</td>
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<td>2.7 and 4.8</td>
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<tr>
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<td>Cryoablation</td>
<td>Ultrasound</td>
<td>≤ T2a</td>
<td>≤ 10</td>
<td>≤ 3 + 4</td>
<td>I</td>
<td>≤ 3 positive biopsies with only 1 lobe involved</td>
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<tr>
<td>2010</td>
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<td></td>
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</tr>
<tr>
<td>2010</td>
<td>HIFU</td>
<td>Ultrasound</td>
<td>≤ T2a</td>
<td>≤ 10</td>
<td>≤ 3 + 4</td>
<td>I</td>
<td>≤ 3 positive biopsies</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2010</td>
<td>Transurethral HIFU</td>
<td>MRI</td>
<td>T1 – T2a</td>
<td>-</td>
<td>≤ 15</td>
<td>≤ 4 + 3</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
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<tr>
<td>2010</td>
<td>HIFU</td>
<td>MRI</td>
<td>≤ T2a</td>
<td>45 – 80 +</td>
<td>≤ 15</td>
<td>≤ 4 + 3</td>
<td>II</td>
<td>Volume ≤ 40 mL or anterior–posterior length ≤ 40 mm</td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
<td></td>
<td>LE of 5 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>Laser therapy</td>
<td>MRI</td>
<td>T1a-T2b</td>
<td>-</td>
<td>≤ 15</td>
<td>≤ 7</td>
<td>II</td>
<td>&lt; 3 positive biopsies.</td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>2010</td>
<td>HIFU</td>
<td>Ultrasound</td>
<td>≥ 45 y</td>
<td>-</td>
<td>≤ 7</td>
<td>I</td>
<td>≤ 3 positive biopsy cores</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
<td>Est. survival ≥ 20 y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2010</td>
<td></td>
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</tr>
<tr>
<td>2010</td>
<td>Radio-Frequency Ablation</td>
<td>Ultrasound or MRI</td>
<td>T2b</td>
<td>-</td>
<td>&lt; 10</td>
<td>≤ 6</td>
<td>I</td>
<td>Size &lt; 60 cc +&lt; 50% cancer in one biopsy + &lt; 25% positive biopsies</td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>HIFU</td>
<td>Ultrasound</td>
<td>T1-T2</td>
<td>45 – 80 y</td>
<td>≤ 10</td>
<td>≤ 7</td>
<td>I</td>
<td>Prostate weight ≤ 40 g</td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Laser therapy</td>
<td>MRI</td>
<td>T1c – T2a</td>
<td>40 – 80 y</td>
<td>&lt; 15</td>
<td>I</td>
<td>≤ 30% cores positive for cancer in sector of pos. biopsy</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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What is still needed to make focal therapy an accepted therapy?
<table>
<thead>
<tr>
<th>Study</th>
<th>Status</th>
<th>Treatment</th>
<th>Stage</th>
<th>Age (y)</th>
<th>Life Expectancy (y)</th>
<th>PSA Density</th>
<th>PSA Velocity</th>
<th>PSA Core %</th>
<th>Lesion Size (mm)</th>
<th>Cancer Visualized</th>
</tr>
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<tr>
<td>Eastham</td>
<td>Ongoing</td>
<td>Cryoablation</td>
<td>T1c-T2a</td>
<td>≥ 21 + LE &gt; 10</td>
<td>≤ 10</td>
<td>≥ 3 + 3</td>
<td>II</td>
<td>II</td>
<td>Size &lt; 60 cc</td>
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</tr>
<tr>
<td>Schoenberg</td>
<td>Ongoing</td>
<td>HIFU / BT</td>
<td>T1c-T2a</td>
<td>LE &gt; 5 y</td>
<td>≤ 10</td>
<td>≤ 6</td>
<td>II/III</td>
<td>I</td>
<td>Size &lt; 40 cc</td>
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</tr>
<tr>
<td>Emberton</td>
<td>Recruiting</td>
<td>IRE</td>
<td>T1-T2c on radiology</td>
<td>LE ≥ 10 y</td>
<td>≤ 15</td>
<td>≤ 7</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morris</td>
<td>Recruiting</td>
<td>BT</td>
<td>T1-T2a</td>
<td>≥ 18 y</td>
<td>&lt; 10</td>
<td>≤ 3 + 4</td>
<td>I</td>
<td>≤ 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emberton</td>
<td>Recruiting</td>
<td>HIFU Ultrasound</td>
<td>T1-T2c and radiological T3a</td>
<td>LE ≥ 10 y</td>
<td>≤ 15</td>
<td>≤ 4 + 3</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinto</td>
<td>Recruiting</td>
<td>Laser therapy MRI</td>
<td>T1c-T2a</td>
<td>&gt; 18 y</td>
<td>≤ 15 or &gt; 15 + PSA</td>
<td>≤ 3 + 4</td>
<td>I/II</td>
<td></td>
<td>Cancer visualized</td>
<td>MRI</td>
</tr>
<tr>
<td>De la Rosette</td>
<td>Recruiting</td>
<td>IRE Ultrasound</td>
<td>Any</td>
<td>LE &gt; 10 y</td>
<td>-</td>
<td>-</td>
<td>I/II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trachtenberg</td>
<td>Recruiting</td>
<td>Laser therapy MRI</td>
<td>T1c-T2a</td>
<td>45 - 80 y + LE &gt; 5</td>
<td>&lt; 15</td>
<td>-</td>
<td>I</td>
<td></td>
<td>MRI confirmed area suspicious for cancer of the positive biopsy</td>
<td></td>
</tr>
<tr>
<td>Zelefsky</td>
<td>Recruiting</td>
<td>BT Ultrasound</td>
<td>T1c-T2a</td>
<td>≥ 21 y + LE &gt; 10 y</td>
<td>&lt; 10</td>
<td>7 in ≤ 2 cores</td>
<td>II</td>
<td>≤ 2</td>
<td>≤ 50 % cancer in one biopsy + ≤ 25 % of cores containing cancer</td>
<td></td>
</tr>
<tr>
<td>InSightec</td>
<td>Recruiting</td>
<td>HIFU MRI</td>
<td>cT1c and cT2a</td>
<td>50 - 75 y</td>
<td>≤ 10</td>
<td>&lt; 3 + 3</td>
<td>I/II</td>
<td></td>
<td>≤ 2 lesions &lt; 10 mm in linear dimension</td>
<td></td>
</tr>
<tr>
<td>InSightec</td>
<td>Recruiting</td>
<td>HIFU MRI</td>
<td>T1c-T2a</td>
<td>55 - 75 y</td>
<td>≤ 10</td>
<td>≤ 6</td>
<td>I</td>
<td></td>
<td>≤ 2 cancer lesions &lt; 10 mm in linear dimension</td>
<td></td>
</tr>
<tr>
<td>Guazzoni</td>
<td>Enrolling</td>
<td>Cryoablation Ultrasound</td>
<td>cT1c - T2a</td>
<td>-</td>
<td>&lt; 10 + PSA vel. &lt; 2ng/ml</td>
<td>≤ 6 +</td>
<td>I</td>
<td></td>
<td>≤ 20 % of pos. cores Imaging: Lesion size &lt; 12 mm</td>
<td></td>
</tr>
<tr>
<td>Polaskic</td>
<td>Not yet recruiting</td>
<td>IRE NR</td>
<td>T1a-T2a</td>
<td>50-70 y</td>
<td>&lt; 10 + PSA dens. &lt; 0.15 + PSA vel. &lt; 2 ng/ml</td>
<td>3 + 3</td>
<td>I</td>
<td></td>
<td>≤20% of cancer in any biopsy core</td>
<td></td>
</tr>
<tr>
<td>Borin</td>
<td>Not yet recruiting</td>
<td>BT Ultrasound</td>
<td>Low risk / Low-volume intermediate risk</td>
<td>&gt; 44 y</td>
<td>&lt; 10</td>
<td>6 or</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ahmed</td>
<td>Unknown</td>
<td>HIFU Ultrasound</td>
<td>≤ T3b</td>
<td>-</td>
<td>≤ 20</td>
<td>≤ 8</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emberton</td>
<td>Unknown</td>
<td>HIFU Ultrasound</td>
<td>≤ T3b</td>
<td>-</td>
<td>≤ 20</td>
<td>≤ 8</td>
<td>II</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GS: Gleason score; US: Ultrasound; BT: brachytherapy; HIFU: high intensity focused ultrasound; IRE: Irreversible electroporation; LDR: low dose rate; y: years; LE: life expectancy; Est.: estimated; PSA vel.: PSA velocity; PSA dens.: PSA density; pos.: positive; NR: Not reported.
TUMOUR FOCI LOCALIZATION

Identifying tumor lesions and targeting these lesions effectively is paramount to achieving success in focal therapy. Furthermore, accurate treatment planning is the key to improved oncological outcomes. To determine the numbers, grade and volume of the tumors and hereby their spatial locations is necessary to make certain that significant cancer is targeted. Therefore, the procedure of taking biopsies and imaging of the prostate has been the focus of recent research.

BIOPSIES

At present, detection of tumour tissue is primarily achieved by systematic transrectal ultrasound (TRUS)-guided biopsies. By altering the systematic approach into targeted TRUS-visible lesion biopsies, a significantly higher cancer-positive rate can be reached. Moreover through delivering the biopsy needles in the center of suspicious hypoechoic lesions, the cancer grade, cancer core percentage and cancer length can vary considerably and perhaps yield significant disease [22]. However, adding MRI-guidance to biopsy results in an improvement in the detection of clinically significant prostate tumors compared to standard biopsy. Retrospective observational studies report that increased prostate tumor detection using image guided techniques can be achieved with less biopsy cores and reduce the detection of insignificant disease [23]. Besides, the use of the Standards of Reporting for MRI-targeted Biopsy Study (START) checklist would improve the quality of reporting in MR-targeted biopsy studies [24]. This checklist also facilitates a comparison between standard and MRI-targeted approaches. Targeting the biopsies guided by MRI-ultrasound fusion did not increase cancer detection [25]. However for patients with prior negative biopsies, MRI-ultrasound fusion biopsy improves the detection of clinically significant tumors [26]. In order to increase the spatial localization of the target lesions for focal treatment, several methods have been investigated. Firstly, extending the amount of biopsies (>20) showed supplementary information on localizing the index lesion and tumor grading [27] However, the number of samples seems more important than the approach by which they are taken, since transrectal and transperineal approaches show similar efficiency [28]. But when altering to the template-assisted transperineal approach, the accuracy increases to 95%, without having a significant negative impact on the quality of life and a negligible complication rate [29] [30]. Despite of the need of general anesthesia or substantial sedation what increases the expenses, it recently became the recommended standard for the selection of patients [5].
These results are limited by their retrospective study design and lack of sample size to achieve statistical power to report significant differences in results.

**IMAGING**

Whereas conventional techniques can only assess anatomical information, more advanced techniques, such as diffusion-weighted imaging (DWI), MR spectroscopy imaging (MRSI), and dynamic contrast-enhanced MRI (DCE-MRI) can obtain functional and metabolic information. A combination of these techniques, referred to as multiparametric MRI (mpMRI), is commonly performed. mpMRI combines, among other sequences, T2MRI for assessing the anatomy, DWI for specifying lesion characteristics, and DCE-MRI for increased cancer detection. Although the value for prostate cancer localization appears to be promising, recent studies are limited by heterogeneous patient populations, variation in inter-reader reliability, inconsistent endpoints, and retrospective study designs. Several new imaging techniques have been investigated to increase the localization of prostate cancer, since conventional imaging techniques have not yet proven sufficiently sensitive for accurate localization. In contrast-enhanced ultrasound, gas-filled micro-sized bubbles are used to visualize the microvascular perfusion of the prostate. The ultrasound waves are inducing the microbubbles to start oscillate and the subsequent backscattered energy can be detected by the ultrasound imaging. Until now, none of the quantification methods using CEUS have yet shown the ability to localize prostate cancer consistently [31]. Elastography is based on quantifying the difference in elastic properties of benign and malignant tissue [32]. Brock et al. combined elastography and CEUS to localize the tumour areas by comparing with whole mount radical prostatectomy histology. This improved the positive predictive value to correctly identify cancer to 89.7% and highlight the possibility of optimizing the visualization of cancer in the future [33]. Shear wave elastography is a comparable system generating a shear wave through prostate tissues, what appears easier to carry out and requires fewer training because it is less subject to operator variability [34]. HistoScanning is a technique that quantifies tissue disorganization induced by malignant processes [35]. In preliminary studies, this method showed a 90% sensitivity and 72% specificity for the localization of lesions ≥ 0.2 mL within a sextant of the prostate [36]. Recently, a multidisciplinary consensus was reached on the role of mpMRI in focal therapy. It recommends performing the mpMRI before prostate biopsies. If this is not possible, a post-biopsy interval period of 6-8 weeks, and preferably greater than 12 weeks, should be regarded to facilitate...
What is still needed to make focal therapy an accepted therapy?

A reliable 5-point scale could be used for communicating the probability of cancer, with a minimum of 16 prostatic regions of interest, to include a pictorial representation of suspicious foci [38].

**IMAGE GUIDANCE DURING TREATMENT**

Focal ablative therapies are mostly performed under transrectal ultrasound guidance (Table 1). However, several MRI-guided platforms have been developed. The MRI guidance can be performed using several methods. The first is called ‘cognitive fusion’; the operator treats the suspicious areas seen on the pre-obtained MRI images with real-time US guidance. The second method is real-time MRI-guided treatments. In this case treatment is performed in an MRI tube. It is yet used with laser therapy and recently with HIFU treatment called Magnetic resonance-guided focused ultrasound (MRgFUS) [8,16]. The third way of MRI guidance is MRI-TRUS fusion. A previously performed MRI is digitally overlaid on real-time ultrasound images. Furthermore, real-time monitoring of thermal focal ablation with MR thermometry appears promising. This non-invasive method of monitoring temperature shows excellent results in canine prostates [39] and is performed in focal laser therapy and transurethral HIFU [40].

**FOLLOW-UP**

Before focal therapy becomes accepted as a viable option, observational studies need mature oncological follow-up data and future study designs require standardization and clear definitions for eligibility criteria and endpoints [18]. Recently, a consensus paper [20] recommended to check post-treatment PSA at three month intervals during the first year, biannually in the second year, and annually in the third year. Thereafter, the frequency of checking PSA is up to the discretion of the investigators. This panel was in agreement that the optimal biopsy strategy includes TRUS-guided systematic whole-prostate biopsies and additionally targeted biopsies, to be taken between 6 and 12 months post-treatment. They state that only in case of clinical suspicion is it advisable to perform biopsies earlier. Pathologists with genitourinary oncology experience are essential to achieve accurate and consistent pathology reports. The biopsy results should be reported in detail, including the number and location of cores taken and the number of positive cores, as well as the amount per biopsy of the involved cancer (in mm). Treatment failure can appointed as in-field
failure and out-of-field failure. In-field failure is defined as biopsy results with a cancer of higher Gleason grade, or persistent cancer of similar or lower grade after repeat focal therapy to the same area, or 3) the need for additional PCa treatment other than focal therapy because of objective findings elsewhere in the gland (e.g., high-grade cancer). Low-grade, low volume tumour foci (< 3mm Gleason 3 + 3) found out-of-field are not designated as failure. Consensus on the role of mp-MRI in focal therapy for prostate cancer states that mp-MRI is the preferred way of focal therapy follow-up with a 95.7% consensus rate. In the face-to-face meeting, the panel agreed that annually mpMRI post-treatment is sufficient. There was a strong agreement that definitive tissue destruction immediately after focal therapy, is presented as loss of enhancement on a DCE MRI. mpMRI should be performed before taking the biopsies (or at least 6-8 weeks afterwards) and should be assessed by trained genitourinary oncology experienced radiologists. This should be pre-determined and described in the protocol of the trial [37]. Furthermore, since the main motivation of focal therapy is to reduce morbidity, it is essential to measure the functional outcomes of the patient using validated patient-reported outcome instruments for sexual, urinary, and bowel function. In addition, the assessment should include measurement quality of life, adverse events and anxiety score. The desirable duration of future prospective phase 2 (single-arm) studies is 18-36 months and prospective phase 3 (randomized) comparative studies is 3 to 5 years. Importantly, the duration of these studies suggest that intermediate endpoints need to be developed and validated which predict cancer-specific outcomes, including metastases and prostate cancer specific-death.

CONCLUSION

In order for focal ablative therapy to be a viable option for the management of localized prostate cancer, it is important to conduct well designed phase I and II trials and proceed forward towards randomized phase III trials. Currently, several phase II trials are in process or will start in the near future. Also some phase III studies are already underway or in preparation. These studies include Active Surveillance versus Focal Therapy treatment using TOOKAD, HIFU versus brachytherapy (conducted by Sonablate), focal-ablation versus extended ablation using IRE (conducted by the Clinical Research Office of the Endourological Society; CROES) and hemi-ablation versus complete ablation using brachytherapy (conducted by ESTRO) [41]. The main focus should be selecting the appropriate patients and establishing thoughtful endpoints demonstrating benefit for treatment. These studies should be
accompanied by new promising imaging techniques providing functional and metabolic information, which will lead to a better visualization and localization of the tumors. Accurate prostate cancer imaging gives the ability to target tumors more precisely and will therefore reduce overtreatment by enabling efficient guidance for focal therapies.
REFERENCES


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29. Hu Y, Ahmed HU, Carter T, et al. A biopsy simulation study to assess the accuracy of several transrectal ultrasonography (TRUS)-biopsy strategies compared with template prostate mapping biopsies in patients who have undergone radical prostatectomy. BJU 2012. 110, 812-820

What is still needed to make focal therapy an accepted therapy?
Chapter 10

Concluding remarks and future prospects
CONCLUDING REMARKS AND FUTURE PROSPECTS

Interesting developments took place in the treatment of prostate cancer including focal therapy for less aggressive organ-confined prostate cancer. Fortunately, curative treatment is often still an option for patients suffering from the lower staged tumors. In carefully selected patients, the prostate cancer may be focally treated followed by careful post-treatment evaluation, and if necessary by focal re-treatment. During the past decades, the age of men at prostate cancer detection has decreased by approximately ten years and men’s life expectancy has increased by almost four years. Together with the increased diagnosis of low- and intermediate risk prostate cancer, interest in the minimally invasive focal treatment with its lower side-effect profile has grown.

Focal therapy is a rapidly evolving field that covers several ablative techniques, energy sources and treatment scenarios. The rationale behind focal therapy sounds relatively simple, targeting the predefined cancerous part of the organ while sparing uninvolved tissue; the execution in prostate cancer is complicated. It is very difficult to predict the patients’ individual clinical development or cancer progression. Selection of the appropriate patient takes into account factors as PSA, biopsy results with histopathological parameters of the cancer foci, patients’ life expectancy and quality of life and most important: the preferences of the patient. After selecting the patient, it remains challenging to localize, visualize and characterize the significant tumour areas and to target the area accurately with the ablative modality most suitable. Finally, after the focal treatment, it is challenging to evaluate treatment efficacy by the interpretation of the serum PSA, imaging- and biopsy results during follow-up.

At present, the different ablative techniques are being studied in early-phase trials, mainly in order to determine the safety of the technique and procedure and to evaluate the efficacy and adverse- and side effects. If the treatment is considered safe and feasible for the targeted ablation of tumorous areas, research will proceed to the next phases. This will include prospective (randomized) controlled trials to determine efficiency, to compare the ablative techniques and finally to test equivalence to current conventional treatments. As written in chapter 2, it is strongly recommended to conduct future research with standardized trial design to create studies with meaningful outcome measures and comparative data.

Additionally, this thesis provides the initial results of IRE research in prostate cancer that show the safety and feasibility of the procedure, quality of life, post-procedural imaging and histopathological outcomes after an IRE-procedure in the prostate. Together with the results of Valerio et al. and Ting et al., the safety and feasibility of
the technique is warranted. Subsequently, larger studies are required to confirm the efficacy in terms of oncological outcomes, patient morbidity and long-term quality of life outcome. A significant improvement of the available evidence on prostate cancer will be established by the Clinical Research Office of the Endourological Society (CROES) coordinated multicenter randomized clinical trial (RCT) on IRE in organ-confined prostate cancer (NCT01835977). This recently started RCT aiming for the inclusion of 200 patients and compares IRE safety and efficacy between a focal ablation and extended ablation of the prostate. Additionally, future studies will involve the ability of increased accuracy of cancer detection, improved imaging techniques as diagnostic tools and enhanced image-guidance for the improvement of tumor targeting during ablation.

Besides studies on IRE in prostate cancer, research into IRE is also focusing on other sort of cancer. Trials on the ablation of liver, pancreas and kidney cancer and furthermore on several unresectable neoplasms tumors are recruiting patients (n=34 on clinicaltrials.gov). Although research into IRE is rapidly growing, most research remains to be in the form of early-phase cohort studies. In order to generate reliable evidence it is a necessity to pool patients and collect data in multicenter registries and to proceed forward to more mature prospective (randomized) controlled trials. Multiple cancer centers are collaborating by pooling data on IRE such as the Soft Tissue Ablation Registry (STAR) registry for pancreatic cancer and the IRE-registry in prostate cancer (NCT02255890) of the CROES.

For focal therapy to evolve into an accepted segment of prostate cancer treatment more research is needed directed at: tissue specific device settings, well-designed clinical trials with standardized ablation protocols, evaluation of short-term ablation results and long-term clinical benefit. In conclusion, research presented in this thesis was just a beginning, there is lots of work to do and the best is yet to come.
Chapter 11

Summary
Focal therapy in prostate cancer

Nederlandse samenvatting
Focale therapie in prostaatkanker
SUMMARY – FOCAL THERAPY IN PROSTATE CANCER

The diagnosis of localized prostate cancer at early stages has increased, because of intensified prostate-specific antigen (PSA) testing and screening, extended prostate biopsy schemes and improved imaging techniques. The average of men’s age at detection of prostate cancer has decreased by approximately 10 years and treatment with curative intent is often well possible. Current radical therapies may be associated with significant side effects impairing patients’ quality of life and are frequently considered as overtreatment for low- or intermediate risk prostate cancers. This has led to increased interest in alternative treatment options.

The present thesis deliberates on a minimally invasive approach of treating prostate cancer within the concept of focal therapy. Focal therapy offers a middle ground between active surveillance and radical treatments as surgery or radiation therapy. Focal therapy is a minimally invasive treatment concept whereby active therapy is targeted to the malignant areas of the prostate gland while sparing the benign tissue and vital structures of the prostate aiming at better preservation of the quality of life.

Chapter 2 describes an international multidisciplinary consensus on trial design for focal therapy in prostate cancer. It provides a standard for designing a feasible focal therapy trial including uniform, systematic pre- and post-treatment evaluation, well defined end points and strict inclusion and exclusion criteria. A four-staged consensus project was conducted, using a modified Delphi method, in which 48 experts in the field of focal therapy of prostate cancer participated. According to this formal consensus-building method, participants were asked to fill out an iterative sequence of questionnaires to collect data on trial design. Subsequently, a consensus meeting was held in which thirteen panellists discussed the obtained data, clarified the results, and defined the conclusions on patient selection, pre-treatment assessment, evaluation of outcome, and follow-up. Inclusion criteria for candidates in focal therapy trials were determined as patients with prostate-specific antigen <15 ng/mL with clinical stage T1c–T2a, Gleason scores 3 + 3 or 3 + 4 and a life expectancy of more than 10 years. It was agreed that the optimal biopsy strategy during follow-up was transrectal ultrasound-guided biopsies to be taken between six months and twelve months after treatment. The primary objective should be focal ablation of clinically significant disease with negative biopsies at twelve months after treatment as the primary end point. Using these recommendations for focal therapy trial design will lead to uniform data acquisition enabling inter-trial comparability.
Chapter 3 evaluates the temperature development and distribution during IRE performed in polyacrylamide gel. Mechanical effects, changes in temperature gradient and absolute temperature changes were measured using three different optical techniques (high speed-, colour Schlieren- and infrared imaging). Effects on temperature by varying voltage, pulse length, active tip length, interelectrode distance, electrode configuration and sequential pulsing were determined. The totally delivered energy was calculated for each procedure. Results show that a temperature gradient develops immediately with pulse delivery, starting at the tips of both electrodes and expanding towards each other. Voltage, pulse length, interelectrode distance, active tip length and electrode configuration have each a strong effect on the temperature development and distribution during IRE ablation, in accordance with higher energy delivery. Non-parallel electrode placement results in heterogeneous temperature distribution with the peak temperature focused in the area with the shortest interelectrode distance. Sequential pulse delivery significantly reduced the temperature increase compared to continuous pulsing and may prove beneficial with respect to procedural safety.

In chapter 4 some consensus statements of chapter 2 were used to design a trial protocol on focal therapy with irreversible electroporation (IRE). Irreversible electroporation is a novel technique using high-voltage direct electric current, travelling between needle-electrodes, for the ablation of tumour tissue. Initially, the manifestation of the irreversible component was considered an unwanted treatment side effect during reversible electroporation. Reversible electroporation has been employed in electrochemotherapy to facilitate the uptake of chemotherapeutic agents into cells. The temporary damage to the cellular membrane allows the chemotherapeutic agent to enter the cell followed by recovery of the membrane. Damage becomes permanent above a certain threshold of electrical pulse length and kV/cm, which causes cell death due to the inability of the cell to maintain homoeostasis. In recent years, interest in IRE as a tumour ablation modality by inducing irreversible cell damage has risen. IRE has shown to be able to effectively ablate tumour cells in vitro in animal experiments and recently in several human safety and efficacy studies for liver, pancreas, kidney and pelvis tumours. Two main factors have fuelled research in IRE as a treatment modality. First, studies in animals and humans have shown that connective tissue structure could be preserved with minor damage to associated blood vessels, neural tissue or other vital structures. Second, IRE lesions show a sharp demarcation between ablated and non-ablated tissue whereas lesions from other ablation techniques often show a transitional zone
containing partially damaged tissue between ablated and healthy tissue, because of partial conduction of heat or cold to the surrounding tissue. The aim of the study is to evaluate the safety and efficacy and to acquire data on patient appreciation of minimally invasive, transperineally image-guided IRE for the focal ablation of prostate cancer. In this multicentre pilot-study, 16 patients with prostate cancer scheduled for radical prostatectomy undergo an IRE procedure, approximately 30 days prior to the radical prostatectomy. The IRE procedures were performed following a focal or extended ablation protocol. Focal ablation consisted of a two or three IRE electrode configuration. Extended ablation consisted of a four or more electrode-configuration. Data as adverse events, side effects, functional outcomes, pain and quality of life are collected before and after the IRE-procedure at several times points. Prior to the IRE procedure and the radical prostatectomy, patients undergo a multiparametric MRI (mpMRI) and contrast-enhanced ultrasound (CEUS) of the prostate. The efficacy of ablation will be determined by comprehensive histopathological examination of the whole-mount sections of the prostate and will be correlated with the electrode configuration and the imaging of the ablation zone.

Chapter 5 aims to compare the volumetric IRE ablation zone on grey-scale transrectal ultrasound (TRUS), CEUS and mpMRI with histopathological findings and to determine the most reliable imaging modality to visualize the IRE effects accurately. 3D-analysis of the ablation volume per prostate was performed on imaging and on H&E stained whole-mount sections. It was demonstrated that on T2-weighted MRI, dynamic contrast enhanced (DCE) MRI and CEUS the effects of IRE were well visible. On grey scale TRUS, the effects were not noticeable. Ablation volume on T2MRI and CEUS closely matched the ablation volume on histopathology. So mpMRI and CEUS are the most feasible imaging modalities to visualize an IRE ablation zone.

In chapter 6 the correlation between the electrode configuration and the histopathology is determined. The area within the electrode configuration, measured on ultrasound, was compared with the area on the H&E stained slide, representing the centre of the ablation zone. We concluded that the histological ablation zone was always ~2.7 times larger than the electrode configuration. Additionally, affected structures such as urethra, capsule, neurovascular bundles were scored per patient and the distance between the structures and nearest electrode were measured and the correlation was determined. Analysis revealed no significant difference between structures close to or at distance to the inserted electrodes.
Chapter 7 evaluates patients’ safety of IRE-procedures and the quality of life following IRE. Mainly mild adverse events occurred during the short-term follow-up, mostly concerning lower urinary tract symptoms. Nearly all resolved between the first and fourth week post treatment. Quality of life assessment showed deterioration in the urinary domain for both treatment protocols. The reported post-procedural pain was low and length of hospital stay was short. So, IRE can be performed safely in patients suffering from prostate cancer. The adverse events are mostly temporary. Quality of life assessment shows deterioration in the urinary domain; however functional outcomes remain stable over time.

The histopathological outcomes of IRE to the prostate and surrounding tissues were described in chapter 8. Following surgical excision of the prostate, the specimen was macroscopically and microscopically examined. Macroscopic examination showed an ellipsoid-shaped haemorrhagic area surrounding small pale discoloured zones at the centre of the IRE ablation zone. The well-demarcated ablation volumes ranged from 5 to 40% of the prostate and extended from the capsule to the prostatic urethra. Microscopic assessment of the ablation zone showed areas of fibrosis without pre-existing glandular ducts, necrosis and ghost-tubules with eosinophilic cytoplasm in 15 out of 16 patients. The area was surrounded by haemorrhage at the location where the electrodes have been located on ultrasound. The ablation zone was well demarcated, showing a trenchant delineation between the viable and non-viable tissue. One prostate specimen showed only fibrosis without a necrotic component. The ablated tissue showed mild to moderate inflammation in all cases with a focal severe component with atrophic cells in one case. Glandular hyperplasia was present in 11 prostates, additionally basal cell hyperplasia and transitional cell metaplasia was identified in 10 and 1 case, respectively. No skip lesions were seen in the area within the electrode configuration. The prostate capsule was affected by the IRE treatment in 12 of 16 cases. IRE effects were observed extending in the neurovascular bundle of 13 patients and the prostatic urethra in 9 patients. Nerve damage was recognized as eosinophilic degeneration of the cytoplasm and pyknotic nuclei of the nerves. Although damage to critical structures adjacent to the prostate occurred, it did not jeopardize the quality of life or functional outcomes of the patients.

Chapter 9 gives a comprehensive overview of the literature on focal treatment modalities for prostate cancer. It shows that it is paramount to extend the amount of biopsies and to alter the way of taking of biopsies to the template-assisted or image-guided approach. Multiparametric MRI is considered the most effective imaging
technique in selecting patients for focal therapy. However, at present, there is still a lack of high-level evidence for the different focal treatment techniques. The early-stage focal therapy trials are conducted including a various selection of patients and different pre-treatment assessment and follow-up, resulting in incomparable data. In order for focal ablative therapy to be a viable option for the management of localized prostate cancer, it is important to conduct well designed phase I and II trials and proceed forward toward randomized phase III trials. Currently, several phase II trials are in process or will start in the near future. Also, some phase III studies are already underway. These studies include active surveillance versus focal therapy treatment using TOOKAD, HIFU versus brachytherapy, focal ablation versus extended ablation using IRE, and hemi-ablation versus complete ablation using brachytherapy. The main focus should be on selecting the appropriate patients and establishing thoughtful endpoints demonstrating benefit for treatment. These studies should be accompanied by promising imaging techniques providing functional and metabolic information, which will lead to a better visualization and localization of the tumours. Accurate prostate cancer imaging and sampling gives the ability to target tumours more precisely, it will therefore reduce overtreatment by enabling efficient guidance for focal therapies.
De diagnose prostaatkanker wordt steeds vaker in een vroeg stadium gesteld. Door prostaatkankerscreening, het frequenter testen van het prostaat-specifiek antigeen (PSA), verbeterde beeldvormende technieken en uitgebreider nemen van prostaatbiopten is de gemiddelde leeftijd bij opsporing van prostaatkanker gedaald met ongeveer tien jaar. In het vroege stadium is een curatieve behandeling van de tumor vaak nog goed mogelijk. De huidige behandelingen (opereren of bestralen) zijn gericht op de gehele prostaat en gaan gepaard met forse bijwerkingen die de kwaliteit van leven van de patiënten negatief kunnen beïnvloeden. Deze radicale behandelingen worden soms beschouwd als overbehandeling voor de prostaattumoren van een laag- en middelrisico. Dit alles heeft ertoe geleid dat de interesse in alternatieve behandelingsopties is gegroeid.

Dit proefschrift gaat over de minimaal invasieve behandeling van prostaatkanker genaamd focale therapie. Focale therapie richt zich alleen op de kwaadaardige gebieden van de prostaat terwijl de goedaardige delen en vitale structuren in de buurt van de prostaat gespaard worden. Focale therapie biedt daarmee een middenweg tussen het actief volgen van de tumor en de conventionele radicale behandelingen, en richt zich op beter behoud van de kwaliteit van leven.

Hoofdstuk 2 beschrijft een onderzoek naar hoe het beste een studie kan worden opgezet naar de focale behandeling van prostaatkanker. De aanbevelingen zijn verkregen door internationale multidisciplinaire consensus van experts op het gebied van focale therapie. Het voorziet in een standaard voor het ontwerpen van een goed uitvoerbare studie, inclusief advies over systematische evaluatie van de patiënt voor en na de behandeling. Het adviseert over strikte inclusie- en exclusiecriteria en goed gedefinieerde eindpunten. Het consensus-project werd uitgevoerd volgens een gemodificeerde Delphi-methode, waaraan 48 deskundigen deelnamen. Volgens deze formele consensusvormende methode, werden de experts gevraagd om antwoord te geven op vragenlijsten betreffende de beste opzet voor studies naar focale therapie in prostaatkanker. Door antwoorden van de groep telkens terug te koppelen aan de deelnemers werd in drie rondes geprobeerd tot consensus te komen. Vervolgens is er een bijeenkomst gehouden waarbij dertien panelleden de verkregen data hebben besproken, conclusies hebben gedefinieerd aldus tot een consensus zijn gekomen. De inclusie criteria voor kandidaten in een focale therapie studie zijn
patiënten met een PSA <15 ng/mL, klinische stagering T1c-T2a en Gleason score 3 + 3 of 3 + 4 en een levensverwachting van meer dan tien jaar. Er werd overeengekomen dat voor oncologische controle de transrectaal echogeleide biopten genomen dienen te worden tussen de zes maanden en twaalf maanden na de behandeling. Het aanbevolen primaire einddoel van de studie is de verwijdering van klinisch significante ziekte, met negatieve biopten na twaalf maanden na behandeling als het primaire eindpunt. Het volgen van deze aanbevelingen zal leiden tot het verkrijgen van beter vergelijkbare data waardoor de verschillende focale technieken beter met elkaar vergeleken kunnen worden.


Gegevens over bijwerkingen, complicaties, pijn, plas- en potentieproblemen en kwaliteit van leven werden verzameld voor en na de IRE-procedure. Voor de IRE-behandeling en voor de prostaatoperatie, ondergingen de patiënten een multiparametrische MRI (mpMRI) en een contrast-echo (CEUS) van de prostaat. Het resultaat van de ablatie werd bepaald door uitgebreid histopathologisch onderzoek en werden gecorreleerd met de plaatsing en het aantal van de elektrodes en de beeldvorming van de ablatiezone.
Hoofdstuk 4 beschrijft een preklinisch onderzoek waarbij IRE-procedures werden uitgevoerd in polyacrylamide gel, waarvan de eigenschappen vergelijkbaar zijn met weefsel. De temperatuurontwikkeling en -gradiënt, warmtedistributie, mechanische effecten en absolute temperatuurveranderingen werden geëvalueerd door middel van drie verschillende optische technieken (high speed-, Schlieren- en infrarood-imaging). De effecten van het variëren van de spanning, puls- en naaldlengte, interelectrode-afstand, elektrode configuratie en sequentiële pulsen (“puls-treintjes”) werden bepaald. De totale geleverde energie werd berekend voor elke procedure. De resultaten toonden direct na de eerste puls afgifte een ontwikkeling van de temperatuurgradiënt, beginnend bij de punt van beide elektroden en zich naar elkaar toe uitbreidend. Voltage, pulslengte, interelectrode-afstand, naaldlengte en elektrode configuratie hadden allen een sterk effect op de temperatuurontwikkeling en -distributie afhankelijk van de totaal geleverde energie. Het bleek dat bij niet-parallel geplaatste elektrodes de temperatuur-distributie niet homogeen was, met een piektemperatuur in het gebied van de kortste electrode-afstand. Tevens bleek dat sequentieel pulsen een significant lagere temperatuurstijging opleverde ten opzichte van continue pulsen, dit kan een gunstig effect hebben op de veiligheid van IRE bij gebruik in de klinische setting.

Hoofdstuk 5 vergelijkt de IRE-ablatiezones op de prostaatecho (TRUS), contrastecho (CEUS) en mpMRI met de histopathologische bevindingen van de zones en onderzoekt wat de meest betrouwbare beeldvormende techniek is om de IRE-effecten te visualiseren. Tevens werd per prostaat een 3D-volume-analyse van het geableerde gebied op de echobezelten en MRI-scan gedaan en vergeleken met het ablatievolume op de histopathologische coupes. Er werd aangetoond dat op de MRI’s (de T2-gewogen en dynamische Contrast MRI) en CEUS de effecten van IRE goed zichtbaar zijn. Op de TRUS echobezelten was dit niet het geval. De ablatie-volumes gemeten op T2MRI en CEUS kwamen goed overeen met de ablatie-volumes op de histopathologische coupes. mpMRI en CEUS zijn daarom de beste beeldvormende modaliteiten om een IRE ablatiezone te visualiseren en te vervolgen.

In hoofdstuk 6 wordt de oppervlakte van het gebied binnen de elektrodes vergeleken met de ablatie-zone op de histopathologische coupes. Ook wordt schade aan de urethra, het prostaatkapsel en aan de neurovasculaire bundels per patiënt gescroond en gecorreleerd aan de afstand tot de meest dichtbijzijnde elektrode. De resultaten toonden dat de ablatie-zone op de coupes ±2,7 keer groter was dan het oppervlaktegebied tussen de naaldelektrodes. Verder werd er geen significant
verschil gevonden tussen de beschadigde en onbeschadigde structuren ten opzichte van de afstand tot de geplaatste elektroden.

**Hoofdstuk 7** evalueert de veiligheid van de IRE-procedures en de kwaliteit van leven na IRE. Tijdens de kortdurende follow-up periode (de vier weken tot de operatie) traden voornamelijk milde bijwerkingen op, zoals geringe klachten van de lage urinewegen en erectiele functie. Deze klachten verdwenen bijna allemaal tussen de eerste en de vierde week na de IRE-behandeling. De kwaliteit van leven toonde een verslechtering op het gebied van urineren, meestal tijdelijk. De gemelde pijn na de procedure was gering en de verblijfsduur in het ziekenhuis was kort (één nacht). Concluderend kan IRE veilig worden uitgevoerd bij patiënten met prostaatkanker.

De histopathologische uitkomsten van IRE in de prostaat en aangrenzende structuren worden beschreven in **hoofdstuk 8**. Na chirurgische excisie van de prostaat werd deze macroscopisch en microscopisch beoordeeld. Macroscopisch onderzoek toonde een ellipsïd-vormige hemorrhagisch gebied rondom een kleine bleke zone in het midden van het de ablatie-zone. De scherp begrenste ablatiezone varieerde van 5 tot 40% van de volume van de prostaat. Microscopische beoordeling toonde uitgebreide fibrose en necrose omgeven door hemorrhagisch gebied in 15 van de 16 prostaten. In het ablatie-gebied werden geen levensvatbare cellen aangetroffen. Ook microscopisch bleek de grenslijn tussen het behandelde weefsel en zijn omgeving scherp te zijn. Eén prostaat toonde fibrose zonder de necrotische component. Het prostaatkapsel was beschadigd door IRE in 12 van 16 patiënten, bij 13 patiënten werd schade aan de neurovasculaire bundel waargenomen en bij 9 patiënten was de urethra ter plaatse van de prostaat aangedaan. Alhoewel er schade optrad aan structuren grenzend aan de prostaat, bleken de afname van kwaliteit van leven en klachten betreffende de lage urinewegen en erectiele functie beperkt.

**Hoofdstuk 9** geeft een uitgebreid overzicht van de literatuur over focale therapie in prostaatkanker. Het laat zien dat het belangrijk is om het aantal prostaatbiopten uit te breiden. Het nemen van de biopten behoort middels een gestandaardiseerd schema en beeld-gestuurd te gebeuren. Tot op heden is de mpMRI de meest effectieve beeldvormende techniek bij de selectie van patiënten voor focale therapie. De onderzoeken naar focale therapie zijn veelal nog vroeg-fase studies en worden uitgevoerd met diverse protocollen, resulterend in onvergelijkbare data. Om focale therapie te ontwikkelen tot een standaard behandelingsoptie van gelokaliseerde prostaatkanker is het van belang goed ontworpen fase I en II studies uit te voeren.
Supplements

Appendix Chapter 2
Author contributions
AMC Graduate School PhD portfolio
List of publications
Curriculum vitae
Dankwoord
Targeted ablation
Hemi-ablation
Zonal ablation
Unilateral nerve-sparing whole-gland ablation
Bilateral nerve-sparing whole-gland ablation

What is focal therapy in prostate cancer?

PSA
PSA velocity
PSA doubling time
PSA density
Free/Total PSA ratio

Which laboratory tests should always be performed to select candidates?

Should MRI-targeted biopsies has to be taken prior to focal therapy?

Ultrasound
MRI
MRI/TRUS fusion

Please select which guidance you recommend to use for systematic biopsies

Ultrasound
MRI
Elastography
Contrast-enhanced ultrasound
MRI/TRUS fusion

Please select which guidance you recommend to use for targeted biopsies:

Is it important to know the amount of tumour involvement in one core biopsy for selecting candidates?

Yes
No
Other
# APPENDIX CHAPTER 2

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<th>Zonal ablation</th>
<th>Unilateral nerve-sparing whole-gland ablation</th>
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### Which questionnaires do you recommend for scoring the erectile function?

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<td>International Index of Erectile Function</td>
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<td>Male Sexual Health Questionnaire</td>
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<td>Sexual Health Inventory for Men</td>
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<td>Quality of Erection Questionnaire</td>
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<td>Erection sufficient for penetration</td>
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### Which questionnaires do you recommend for scoring urinary symptoms?

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<td>International Prostate Symptom Score</td>
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<td>International Continence Society Scale</td>
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<td>Urgency, Weak stream, incomplete emptying and nocturia instrument</td>
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<td>Continence questionnaire</td>
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### Do you recommend to score the use of pads additionally to the IPSS in scoring urinary symptoms?

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<td>Score pads</td>
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### Which questionnaire for quality of life should be used?

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<td>Expanded Prostate Cancer Index Composite (EPIC)</td>
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<td>Functional Assessment of Cancer Therapy-Prostate (FACT-P)</td>
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### Which of the imaging techniques do you recommend to optimize preplanning of treatment?

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<td>Histoscanning</td>
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<td>Acoustic radiation force impulse imaging</td>
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Which inclusion criteria should candidates meet?

### Initial PSA level of:

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### Initial PSA density of:

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### TNM stage from:

<table>
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<th>T1a</th>
<th>T1b</th>
<th>T1c</th>
<th>T2a</th>
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<tbody>
<tr>
<td>9</td>
<td>7</td>
<td>70</td>
<td>14</td>
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### TNM stage till:

<table>
<thead>
<tr>
<th>T2a</th>
<th>T2b</th>
<th>T2c</th>
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</thead>
<tbody>
<tr>
<td>60</td>
<td>27</td>
<td>13</td>
</tr>
</tbody>
</table>

### Gleason score of:

- **3 + 3**: 100
- **3 + 4**: 95
- **4 + 3**: 60
- **4 + 4**: 16
- **4 + 5**: 5
- **5 + 4**: 5
- **5 + 5**: 5

### Life expectancy of:

<table>
<thead>
<tr>
<th>≥ 5 years</th>
<th>&gt; 10 years</th>
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</thead>
<tbody>
<tr>
<td>7</td>
<td>77</td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>This should not be an inclusion criterium</td>
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</table>
### Minimum of age:

- 45 years
- 50 years
- 60 years

This should not be an inclusion criterium.

### Maximum of age:

- 75 years
- 80 years
- 85 years

This should not be an inclusion criterium.

### Maximum of prostate size

- 50 mL
- 70 mL

Depends on ablative technique.

This should not be an inclusion criterium.

### In case of 10 taken biopsies per lobe, patients should be included with:

- Unilateral disease ≤ 2 positive biopsies
- Unilateral disease ≤ 3 positive biopsies
- Unilateral disease ≤ 5 positive biopsies
- Unilateral disease ≤ 6 positive biopsies
- Bilateral disease
- Other

### Is MRI-visible lesion concordancy with biopsy mandatory for inclusion?

- Yes, at least 1
- This should not be an inclusion criterium

### Should 5α-reductase inhibitors be stopped before a focal therapy trial?

- Yes
- No
- Other

### What is the preferred catheterization method?

- Urethral
- Suprapubic
- Other
### Which exclusion criteria should candidates meet?

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Yes (%)</th>
<th>No (%)</th>
<th>Other (%)</th>
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<tbody>
<tr>
<td>Extracapsular extension on radiology</td>
<td>82</td>
<td>15</td>
<td>3</td>
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<tr>
<td>Lymph node or bone metastasis</td>
<td>97</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>20</td>
<td>80</td>
<td>92</td>
</tr>
<tr>
<td>Active urinary tract infections</td>
<td>23</td>
<td>77</td>
<td>32</td>
</tr>
<tr>
<td>History of acute or chronic prostatitis within the past 2 years</td>
<td>66</td>
<td>32</td>
<td>2</td>
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<tr>
<td>Congestive heart failure, myocardial infarction or other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant erectile dysfunction</td>
<td>85</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Urinary incontinence</td>
<td>41</td>
<td>55</td>
<td>27</td>
</tr>
<tr>
<td>Diagnosed with cancer that is not considered cured, except basal...</td>
<td>66</td>
<td>34</td>
<td>50</td>
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<tr>
<td>Prior transurethral resection of the prostate with a large tissue defect</td>
<td>46</td>
<td>50</td>
<td>4</td>
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<tr>
<td>Significant symptoms of urinary obstruction prior to treatment</td>
<td>57</td>
<td>43</td>
<td></td>
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<tr>
<td>History of abdominoperineal resection for rectal cancer, rectal...</td>
<td>64</td>
<td>30</td>
<td>4</td>
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<tr>
<td>Contra-indication to MRI</td>
<td>37</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Previous prostate cancer treatment as androgen...</td>
<td>50</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>Previous radiation therapy or androgen suppression/hormone...</td>
<td>48</td>
<td>52</td>
<td>7</td>
</tr>
<tr>
<td>Any previous treatment for prostate cancer</td>
<td>30</td>
<td>68</td>
<td>2</td>
</tr>
</tbody>
</table>

### Should androgen deprivation therapy be stopped?

- Yes: 97%
- No: 2%
- Other: 1%

### Should 5α-reductase inhibitors be stopped before a focal therapy trial?

- Yes: 39%
- No: 59%
- Other: 2%

### What is the preferred catheterization method?

- Urethral: 82%
- Suprapubic: 10%
- Other: 8%

### Do you recommend the use of antibiotic prophylaxis?

- Yes: 95%
- No: 5%
- Other: 0%
### What would you recommend to perform in follow-up?

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<tr>
<th>Test</th>
<th>Yes</th>
<th>No</th>
<th>Other</th>
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<tbody>
<tr>
<td>PSA</td>
<td>100</td>
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<tr>
<td>PSA derivates</td>
<td>45</td>
<td>55</td>
<td>3</td>
</tr>
<tr>
<td>Biopsies</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary symptom score</td>
<td>97</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Erectile function score</td>
<td>95</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Quality of life questionnaire</td>
<td>97</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Anxiety-score</td>
<td>66</td>
<td>37</td>
<td>7</td>
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<tr>
<td>Adverse events</td>
<td>95</td>
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### Which imaging modalities do you recommend for focal therapy follow-up

<table>
<thead>
<tr>
<th>Modality</th>
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<th>No</th>
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<tr>
<td>TRUS</td>
<td>59</td>
<td>40</td>
<td>7</td>
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<tr>
<td>(mp)MRI</td>
<td>2</td>
<td></td>
<td>1</td>
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<tr>
<td>HistoCT scan</td>
<td>8</td>
<td>92</td>
<td></td>
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<tr>
<td>LD planning</td>
<td>3</td>
<td>87</td>
<td>10</td>
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</table>

### Control of PSA levels in follow-up should be performed:

<table>
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<tr>
<th>Time Period</th>
<th>Yes</th>
<th>No</th>
<th>Other</th>
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<tbody>
<tr>
<td>First year post-treatment</td>
<td>95</td>
<td></td>
<td>5</td>
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<tr>
<td>Second year post-treatment</td>
<td>13</td>
<td>79</td>
<td>8</td>
</tr>
<tr>
<td>Third year post-treatment</td>
<td>9</td>
<td>71</td>
<td>18</td>
</tr>
<tr>
<td>&gt; Third year post-treatment</td>
<td>42</td>
<td>56</td>
<td>2</td>
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</table>

### What do you designate as treatment failure?

<table>
<thead>
<tr>
<th>Failure Type</th>
<th>Yes</th>
<th>No</th>
<th>Other</th>
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<tbody>
<tr>
<td>In-field disease</td>
<td>97</td>
<td></td>
<td>3</td>
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<tr>
<td>Out-of-field disease</td>
<td>30</td>
<td>70</td>
<td>5</td>
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<tr>
<td>When there is a need for additional Pca treatment other than focal therapy</td>
<td>77</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>When there is a need for additional Pca treatment</td>
<td>70</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>
What is an accurate definition for biochemical failure in focal therapy?

- American Society for Therapeutic Radiology and Oncology Criteria
- Phoenix criteria
- PSA doubling time
- PSA velocity
- This should be defined differently
- Biochemical failure should not be used in focal therapy trials

In case of treatment failure, what do you recommend as retreatment?

- Again focal therapy
- Radical prostatectomy
- Depends on patient preference
- Depends on characteristics of recurrence

If negative biopsies is an endpoint of your trial, when should it be reached?

- 6 months post-treatment
- 12 months post-treatment

What do you recommend as minimal duration of follow-up?

- 1 year
- 2 years
- 3 years
- 5 years
- 10 years
- Other

In case of stratified randomization in a RCT, which of the following classifications should be used for stratifying the patients?

- TNM classification
- d'Amico risk classification
- ESTRO-EAU classification
- UCSF-CAPRA score
- Partin tables
- Kattan nomograms
- Prostate Px+
- Task Force Focal Prostate Cancer Patient selection criteria

- Yes
- No
- Other
AUTHOR CONTRIBUTIONS

Chapter 2
Focal therapy in prostate cancer: International multidisciplinary consensus on trial design

W van den Bos, BG Muller, HU Ahmed, CH Bangma, E Barret, S Crouzet, SE Eggener, IS Gill, S Joniau, G Kovacs, S Pahernik, O Rouviere, G Salomon, JJMCH de la Rosette, MR Meijerink, JH Scheffer, RM Verdaasdonk, JJ Klaessens, PS Zondervan

Conception and design: WB, BM, DB, JR and PS
Data/literature acquisition: WB and BM
Data/literature analysis and interpretation: WB, BM, PE, JL and PS
Drafting the manuscript: WB, BM, DB, JL and PS
Critical revision of the manuscript: DB, JR and PS
Supervision: DB, JR and PS

Chapter 3
Thermal energy during irreversible electroporation and the influence of different ablation parameters

W van den Bos, HJ Scheffer, JA Vogel, PGK Wagstaff, DM de Bruin, MJ van Gemert, JJMCH de la Rosette, MR Meijerink, JH Klaessens, RM Verdaasdonk

WB and HS have equally contributed to the manuscript
Conception and design: WB, HS, JV, DB, JK and RV
Data acquisition: WB, HS, JV, DB, JK and RV
Data analysis and interpretation: WB, HS, JK and RV
Chapter 4
The safety and efficacy of irreversible electroporation for the ablation of prostate cancer: A multicentre prospective human in vivo pilot study protocol

W van den Bos, DM de Bruin, BG Muller, IM Varkarakis, AA Karagiannis, PJ Zondervan, MP Laguna Pes, DP Veelo, CD Savci-Heijink, MRW Engelbrecht, H Wijkstra, TM de Reijke, JJMCH de la Rosette

Conception and design: WB, BM, DB, DV, CS, TR and JL
Data/literature acquisition: WB, DB and BM
Data/literature analysis and interpretation: WB, DB and BM
Drafting the manuscript: WB and DB
Critical revision of the manuscript: All authors
Supervision: TR and JL

Chapter 5
MRI and contrast-enhanced ultrasound imaging for evaluation of focal irreversible electroporation treatment: Results from a phase I-II study in patients undergoing IRE followed by radical prostatectomy

W van den Bos, DM de Bruin, A van Randen, MRW Engelbrecht, AW Postema, BG Muller, IM Varkarakis, AS Skolarikos, CD Savci-Heijink, RR Jurhili, PJ Zondervan, MP Laguna Pes, H Wijkstra, TM de Reijke, JJMCH de la Rosette

Conception and design: WB, DB, BM and JR
Data acquisition: WB, AP, BM, IV, AS, PZ, ML, TR and JR
Data analysis and interpretation: WB, DB, AR, ME, CS, RJ and HW
Statistical analysis: WB and DB
Drafting the manuscript: WB and DB
Critical revision of the manuscript: All authors
Supervision: ME and JR
Chapter 6
The correlation between the electrode configuration and histopathology of irreversible electroporation ablations in prostate cancer patients
W van den Bos, DM de Bruin, RR Jurhill, CD Savci-Heijink, BG Muller, IM Varkarakis, A Skolarikos, PJ Zondervan, MP Laguna Pes, H Wijkstra, TM de Reijke, JJMCH de la Rosette

Conception and design: WB, BM, DB, and JR
Data/literature acquisition: WB, DB, JR, CS, BM, IV, AS, PZ, ML, TR and JR
Data analysis and interpretation: WB, DB, RJ, and CS
Statistical analysis: WB and DB
Drafting the manuscript: WB and DB
Critical revision of the manuscript: All authors
Supervision: TR, JL

Chapter 7
Quality of life and safety outcomes following irreversible electroporation treatment for prostate cancer: results from a phase I-II study
W van den Bos, DM de Bruin, DP Veelo, AW Postema, BG Muller, IM Varkarakis, A Skolarikos, PJ Zondervan, MP Laguna Pes, CD Savci-Heijink, H Wijkstra, TM de Reijke, JJMCH de la Rosette

Conception and design: WB, DB, DP, BM and JR
Data acquisition: WB, DV, AP, BM, IV, AS, PZ, ML, TR and JR
Data analysis and interpretation: WB, AP and DB
Statistical analysis: WB and DB
Drafting the manuscript: WB
Critical revision of the manuscript: All authors
Supervision: DB, JR
Chapter 8
Histopathological outcomes after irreversible electroporation in prostate cancer
W van den Bos, RR Jurhill, DM de Bruin, CD Savci-Heijink, AW Postema, PGK Wagstaff, BG Muller, IM Varkarakis, A Skolarikos, PJ Zondervan, MP Laguna Pes, TM de Reijke, JJMCH de la Rosette

Conception and design: WB, DB, BM
Data acquisition: WB, AP, BM, IV, AS, PZ, ML, TR and JR
Data analysis and interpretation: WB, RJ, CS and JR
Drafting the manuscript: WB and RJ
Critical revision of the manuscript: All authors
Supervision: CS and JR

Chapter 9
What is still needed to make focal therapy an accepted segment of standard therapy?
W van den Bos, BG Muller, B Ehdaie, P Scardino, JJMCH de la Rosette

Conception and design: WB and BM
Data/literature acquisition: WB and BM
Data/literature analysis and interpretation: WB, BM and BE
Drafting the manuscript: WB, BM and BE
Critical revision of the manuscript: All authors
Supervision: PS, JR
AMC GRADUATE SCHOOL PhD PORTFOLIO

Portfolio Willemien van den Bos
PhD period September 2012 – May 2016
Location Department of Urology and Biomedical Engineering and Physics
Academic Medical Center, Amsterdam
PhD supervisors Prof. dr. JJMCH de la Rosette, prof. dr. AGJM van Leeuwen
Co-supervisors Prof. dr. MP Laguna Pes, dr. TM de Reijke, dr. DM de Bruin

PhD training

<table>
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<tr>
<th>Graduate School Courses</th>
<th>Year</th>
<th>ECTS</th>
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<tbody>
<tr>
<td>Entrepreneurship in Health and Life Sciences</td>
<td>2015</td>
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<td>Oral Presentation in English</td>
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<td>Practical Biostatistics</td>
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<tr>
<td>Basic Course in Legislation and Organization for Clinical Researchers</td>
<td>2013</td>
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<td>Clinical Data Management</td>
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<tr>
<td>The AMC World of Science</td>
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<td>Web of Science</td>
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Oral, poster and video presentations

<table>
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<tr>
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<tr>
<td>Synergy 2015, a multidisciplinary approach to interventional oncology, Miami. Oral presentation</td>
<td>2015</td>
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<td>IRE in Prostate Cancer: Update on the CROES Trial</td>
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<td>MRI and CEUS imaging for evaluation of IRE treatment: results from a phase I/II study in prostate cancer patients undergoing IRE followed by radical prostatectomy. Moderated ePoster</td>
<td>2015</td>
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<tr>
<td>The correlation between treatment planning and histopathology of irreversible electroporation ablations in prostate cancer patients. Moderated ePoster</td>
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<tr>
<td>Quality of life and safety outcomes of irreversible Electroporation for prostate cancer. ePoster</td>
<td></td>
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<tr>
<td>8th International Symposium on Focal Therapy and Imaging in Prostate &amp; Kidney Cancer. IRE symposium – CROES phase I study. Oral presentation</td>
<td>2015</td>
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<tr>
<td>MRI and CEUS imaging for evaluation of IRE treatment: results from a phase I/II study in prostate cancer patients undergoing IRE followed by radical prostatectomy. Oral and poster presentation</td>
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<td>The correlation between the electrode configuration and histopathology of irreversible electroporation ablations in prostate cancer patients. Poster presentation</td>
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<td>Quality of life and safety outcomes following irreversible electroporation treatment for prostate cancer. Poster presentation</td>
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### (Inter)national conferences, seminars, masterclasses

<table>
<thead>
<tr>
<th>Conference</th>
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<tr>
<td>Synergy 2015, multidisciplinary approach to interventional oncology, Miami, USA</td>
<td>2015</td>
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<td>35th Congress of the Société Internationale d’Urologie, Melbourne, Australia</td>
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<td>8th Intern. Symposium on Focal Therapy &amp; Imaging, Noordwijk, The Netherlands</td>
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<td>30th Annual EAU Congress, Madrid, Spain</td>
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<td>7th Intern. Symposium on Focal Therapy &amp; Imaging, Pasadena, United States</td>
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<tr>
<td>29th Annual EAU Congress, Stockholm, Sweden</td>
<td>2014</td>
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<td>6th Intern. Symposium on Focal Therapy &amp; Imaging, Noordwijk, The Netherlands</td>
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<tr>
<td>1st International “State-of-the-Art” Conference on Prostate and Kidney Cancers, Amsterdam, The Netherlands</td>
<td>2013</td>
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<td>Synergy 2013, multidisciplinary approach to interventional oncology, Miami, USA</td>
<td>2013</td>
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<td>31th World Congress of Endourology, New Orleans, USA</td>
<td>2013</td>
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<tr>
<td>7th YUP symposium, the Netherlands</td>
<td>2012</td>
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<tr>
<td>Masterclass Metastasized Prostate cancer, Amsterdam, the Netherlands</td>
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<td>18th - 20th AUA review, the Netherlands</td>
<td>'12 - '14</td>
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<tr>
<td>16th – 18th EAU review, the Netherlands</td>
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<td>Journal clubs AMC and VUmc Amsterdam, the Netherlands</td>
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### Parameters of Esteem

<table>
<thead>
<tr>
<th>Category</th>
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<tr>
<td><strong>Best poster awards</strong></td>
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<tr>
<td>35th Congress of the Société Internationale d’Urologie, Melbourne, Australia</td>
<td>2015</td>
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<tr>
<td>8th International symposium on Focal Therapy and Imaging in Prostate &amp; Kidney Cancer, Noordwijk, The Netherlands</td>
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<td><strong>Nominations for best poster award</strong></td>
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<tr>
<td>30th Annual EAU Congress 2015, Madrid, Spain</td>
<td>2015</td>
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<td>29th EAU Congress, Stockholm, Sweden</td>
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<td>World Congress of Brachytherapy 2012, Barcelona, Spain</td>
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<tr>
<td>Cure for Cancer</td>
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</table>
LIST OF PUBLICATIONS

Focal vs Extended ablation in localized prostate cancer with irreversible electroporation; A Multi-Center Randomized Controlled Trial
MJV Scheltema*, W van den Bos*, DM de Bruin, H Wijkstra, MP Laguna Pes, TM de Reijke, JJMCH de la Rosette
   BMC Cancer – submitted

Surveillance following focal therapy interventions
AW Postema, M Catellani, MJV Scheltema, W van den Bos, JJMCH de la Rosette
   Archivos Espanoles de Urologia – submitted

Irreversible electroporation, a new modality in focal therapy for prostate cancer
MJV Scheltema, W van den Bos, PGK Wagstaff, AW Postema, DM de Bruin, MP Laguna Pes, JJMCH de la Rosette
   Archivos Espanoles de Urologia – submitted

Histopathological outcomes after irreversible electroporation in prostate cancer
W van den Bos, RR Jurhill, DM de Bruin, CD Savci-Heijink, AW Postema, PGK Wagstaff, BG Muller, IM Varkarakis, A Skolarikos, PJ Zondervan, MP Laguna Pes, TM de Reijke, JJMCH de la Rosette
   Journal of Urology – in press

The influence of a metal stent on the distribution of thermal energy during irreversible electroporation
HJ Scheffer, JA Vogel, W van den Bos, RE Neal II, KP van Lienden, MGH Besselink, MJC van Gemert, CWM van der Geld, MR Meijerink, JH Klaessens, RM Verdaasdonk
   Plos One – in press

Irreversible electroporation: state-of-the-art
PGK Wagstaff, M Buijs, W van den Bos, DM de Bruin, PJ Zondervan, JJMCH de la Rosette, MP Laguna Pes
   OncoTargets and Therapy – in press
Standardization in definitions in focal therapy in prostate cancer: report from a Delphi consensus project
World J Urol. 2016 Feb 18. (Epub ahead of print)

Customized tool for validation of optical coherence tomography in differentiation of prostate cancer
BG Muller, A Swaan, DM de Bruin, W van den Bos, AW Schreurs, DJ Faber, ECH Zwartkruis, L Rozendaal, AN Vis, JA Nieuwenhuijzen, RJA van Moorselaar, TG van Leeuwen, JJMCH de la Rosette
Technol Cancer Res Treat. 2016 Jan 27. (Epub ahead of print)

Randomized controlled trial on irreversible electroporation for localized prostate cancer: focal ablation versus extended ablation
W van den Bos W, JJ de la Rosette
J Endourol. 2015 Aug;29(8):851-4

Quality of life and safety outcomes following irreversible electroporation treatment for prostate cancer: results from a phase I-II study
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List of presentations

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Oral presentation
  *IRE in Prostate Cancer: Update on the CROES Trial*

35th Congress of the Société Internationale d’Urologie 2015, Melbourne, Australia
Oral presentations and poster presentations
  *Quality of life and safety outcomes of Irreversible Electroporation for prostate cancer*
  *MRI and CEUS imaging for evaluation of ire treatment: results from a phase I/II study prostate cancer patients undergoing IRE followed by radical prostatectomy*
  *The correlation between treatment planning and histopathology of irreversible electroporation ablations in prostate cancer patient*
  Winner Best poster award

8th International Symposium on Focal Therapy & Imaging in Prostate & Kidney cancer 2015, Noordwijk, the Netherlands
Oral and poster presentations
  *IRE symposium – CROES phase I study*
  *The correlation between the electrode configuration and histopathology*
of irreversible electroporation ablations in prostate cancer patients
Quality of life and safety outcomes following irreversible electroporation treatment for prostate cancer
MRI and CEUS imaging for evaluation of IRE treatment: results from a phase I/II study in prostate cancer patients undergoing IRE followed by radical prostatectomy
Winner Best Poster Award

30th Annual EAU Congress 2015, Madrid, Spain
Oral and poster presentation
Imaging of the ablation zone after focal irreversible electroporation treatment in prostate cancer
Nominated for Best Poster award.

7th International Symposium on Focal Therapy & Imaging in Prostate & Kidney Cancer 2014, Pasadena, USA
Poster presentations + Poster discussion sessions
Irreversible electroporation effects assessed on 3D-histopathology of the prostate
Irreversible electroporation of the porcine kidney: Temperature development and distribution

29th Annual EAU Congress 2014, Stockholm, Sweden
Poster presentation + Poster discussion session
From Gleason score to changes in scattering: Optical coherence tomography in prostate cancer – a prospective human ex-vivo study
Focal Therapy in Prostate Cancer: International Multidisciplinary Consensus on Trial Design
Nominated for Best Poster in poster discussion session

World Congress of Brachytherapy 2012, Barcelona, Spain
Poster presentation + Poster discussion session
Dose contribution to involved pelvic nodes with 3D brachytherapy for cervical cancer; a comparison of two cancer centers
Nominated for Best Poster Award
CURRICULUM VITAE

Willemien van den Bos was born in Apeldoorn, The Netherlands on April 11th, 1987. She was raised together with her siblings in a happy and supportive family. She graduated from Gymnasium Apeldoorn in 2005 and continued with medical school at the University of Utrecht. During the course of her studies, she completed two clinical internships abroad, at the Kasturba Medical College in Manipal, India and at Stichting Wit Gele Kruis in Willemstad, Curaçao. She obtained her medical degree in 2012 after a research rotation on radiation therapy in cervical cancer at Magees Womens hospital, University of Pittsburgh Medical Center in Pittsburgh, PA, USA.

In September 2012 she started working as research physician at the department of Urology at the Academic Medical Center, Amsterdam. She worked under the supervision of prof. dr. J.J.M.C.H. de la Rosette and prof. dr. A.G.J.M. van Leeuwen on multiple studies concerning focal therapy in prostate cancer. The results of her research are presented in this thesis.

Willemien is currently appointed as research fellow at the Garvan Institute of Medical Research in Sydney, Australia.
DANKWOORD

Na mijn afstuderen, moedigde mijn vader me aan om me te verdiepen in de wondere wereld van de wetenschap. Het zou me de kans geven om mijzelf verder te ontwikkelen en het zou waarde toevoegen aan mijn carrière. Bovendien zou ik ‘het gewone dokteren’ de rest van mijn leven nog kunnen doen. Ik ben ervoor gegaan en hij heeft op alle punten gelijk gehad… Voor u ligt het resultaat!

Om te beginnen wil ik de patiënten die deel hebben genomen aan de studie heel hartelijk bedanken. Zonder u was dit belangrijke en fundamentele onderzoek naar IRE en ook dit proefschrift er nooit geweest.

Promotoren:
Professor de la Rosette, beste prof. Ik wil u heel graag bedanken voor al uw wijsheid, medische kennis en organisatorische inzicht. U heeft me daarvan de afgelopen jaren veel bijgebracht en u heeft bovendien altijd vertrouwen in mij gehad. U heeft mij rechtstreeks uit de collegebanken aangenomen en me altijd het gevoel gegeven dat ik dit kon. Het is bewonderenswaardig hoe u kliniek, wetenschap, (inter)nationaal congresbezoek en -organisatie met elkaar weet te combineren. Daarnaast kreeg ik mijn manuscripten razend snel volledig gereviseerd van u terug. Waar haalt u de tijd vandaan? U heeft mij meerdere malen de mogelijkheid gegeven om de studieresultaten te presenteren op internationale congressen. Daar heb ik niet alleen veel van geleerd, maar daar heb ik ook veel contacten opgedaan en meestal kon ik er ook nog een mooie vakantie aan vastplakken! Ook daarvoor dank :)
Professor van Leeuwen, beste Ton. Ik wil je bedanken voor je oprechte interesse in mijn onderzoek en zo af en toe een scherpe blik op de voortgang ervan. Je runt een waanzinnig motiverende afdeling die zoveel waardevolst toevoegt aan de kliniek en dat doe je altijd met een lach. Het was een feest om op je afdeling te mogen werken.

Copromotoren: Professor Laguna en Dr. de Reijke. Veel dank voor uw kritische en grondige blik op al mijn manuscripten. Uw urologische kennis van de literatuur en kliniek is enorm en zeer waardevol. Ik heb heel veel van u geleerd, waarvoor veel dank.

Dan, Dr. Daniel Martijn de Bruin, DMDB, Martino. Hoe onwijs gaaf dat jij mijn copromotor bent en wat heb ik veel aan jou te danken! Drie jaar lang heb je aan mijn zijde gestaan en zonder jou bestond dit boek niet. Heel veel cappuccino’s, blikjes cola...
en dropzakken hebben we samen verorberd om de Excel-files om te zetten in mooie platen! En Eva, dank voor je begrip, als Martijn weer eens te laat thuis kwam... Bruno, ik zal de congressen samen nooit vergeten. Wat hebben we een aantal onwijs mooie trips gemaakt: New Orleans, Californië, Berlijn, Madrid en natuurlijk Noordwijk niet te vergeten! Lang leve de wetenschap :)

De overige leden van de promotiecommissie, Prof. Verdaasdonk, Prof. van Moorselaar, Prof. Pelger, Prof. van de Vijver, Prof. van Delden, Dr. Faber en Dr. Besselink. Hartelijk dank voor het beoordelen van mijn proefschrift en de bereidheid zitting te nemen in mijn promotiecommissie.

Mijn grote dank gaat ook uit naar stichting Cure for Cancer, die mijn promotietraject grotendeels financieel mogelijk heeft gemaakt.

Kamertje 249, the cradle of greatness, Arnoud en Peter, hoe kan ik jullie bedanken? Wat heb ik onwijs veel en vaak met jullie gelachen! Betere kamergenoten zou ik me niet hebben kunnen wensen. Drie jaar lang op elkaars lip op die 4 m2 en er is nooit één onvertogen woord gevallen. Ik ga jullie onwijs missen; de eindeloze grappen en rollen over Perzische P-prinsessen en S-taart-dilemma’s... Maar ook de uurtjes knallen met stroopwafels, drop-fruit duo’s en techno-deuntjes op de achtergrond. Veel dank ook voor het inzicht dat jullie me gegeven hebben in het mannelijk brein: dat zit uiteindelijk toch best eenvoudig in elkaar!

Peet -God of Kidneys- Wagstaff, wat was het een feest om naast jou te mogen zitten. Je eindeloze kennis over van álles en nog wat, de mooiste verhalen over wat je vroeger wel niet allemaal hebt uitgespookt en je inzicht in de Engelse taal hebben mijn promotietijd een stuk draaglijker, maar vooral ook heel veel leuker gemaakt. De congressen samen waren top en de trip door Californië was briljant. Ik vind het waanzinnig dat wij samen op dezelfde dag gaan promoveren. Dankjewel dat je mijn paranimf wilt zijn! Als ik de antwoorden niet weet, dan weet ik zeker dat ik op jou kan rekenen!

Arnie -DEUS of CEUS- Postema, bedankt voor je vrolijkheid, je enthuasiaste en het lachen! Mooi om te zien hoe fanatiek jij kan zijn als je ergens voor gaat, op professioneel gebied maar zeker ook daarbuiten. En bijzonder om te zien hoe jouw mood afhankelijk is van het aantal uur dat je slaapt! Trots dat wij samen Peetjes paranimfen mogen zijn! Nu jij ook gauw je boek afmaken en zo snel mogelijk professor Postema worden.

Dan al mijn urologie collega’s! De urologie-dames, Martine, Laura, Natasha, Alice, Sonja, Nienke en Aletta. Veel succes, maak er wat moois van! En mijn ‘opvolger’ Matthijs. Je kan het! Make us proud!

Dan de collega’s van beneden… Sanne, Abel, Mitra, Anouk, Dirk, Paul, Martin vG, Martin B. Bedankt voor de gezellige tijd maar ook voor het meedenken bij de maandagochtend-meeting! Jullie zijn een bijzonder waardevolle afdeling. En OCT is oké, olé olé!

AMC-ers van alle andere afdelingen, bedankt voor de gezelligheid op het Voetenplein! Hannah, hoeveel cappu’s hebben wij niet gedronken waarbij we onze weekenden en de ups- en downs van promoveren doornamen? Dankjewel daarvoor! Ik kijk nu al uit naar jouw proefschrift!

Leo, net AMC-af, maar wat was het leuk om samen met jou in één ziekenhuis te werken, jij met drie piepers op zak en ik met alle tijd. Ik bewonder het hoe je alles zo goed combineert. Je bent een groot voorbeeld voor me.

Lotje, Paul, Janneke, Sophie, Meike, Nienke, Anne-Fre en iedereen die ik vergeet, thanks voor de gezelligheid! It was a blast!

Martino en Hes, wat ben ik blij dat ik jullie via de Nanoknife heb leren kennen. Wat een topgroep is het om mee op stap te gaan en congressen mee te bezoeken! Miami 2016, here we come!

Co-auteurs van de pathologie: Dilara en Roy, dank jullie wel voor alle moeite en tijd die jullie hebben gestoken in de analyse van de IRE-coupes. Zonder jullie was dit onderzoek niet geslaagd! Co-auteurs van de radiologie: Marc en Adrienne, dank voor het analyseren van de MRI’s, ik hoop jullie toekomstig collega te worden!

Dan ál mijn lieve vriendinnen/jjes; -van vroeger (WLFTP, SopGus), -Bikkels (Lot, Joop, IJk, Bib en de rest) -Wijde Willempies (Jos, Leo, Ger, Ted, Rob, Henk, Gijs, Karel, Ruud, Wim en de rest!) -Roomies van Prinsengracht 180 (Willemijn&Lisa), Hurley dames 5 en iedereen die ik nu vergeet! Heel veel dank voor het luisteren naar mijn prostaatverhalen, bedankt voor de leuke feestjes, diners, verjaardagen en huwelijken die ik met jullie heb mogen meemaken en hopelijk nog ga meemaken!

Lieve broer en zus, Toon en Hanneke. Wat ben ik blij dat ik jullie kleine zusje ben! Het is heerlijk om te weten dat jullie er altijd zijn om mee te denken, hulp te bieden als het kan en advies te geven waar nodig. Het is waanzinnig fijn om zo’n sterke band met jullie te hebben.

Toon, ik vind het geweldig hoe keihard en gefocust jij kan werken, daarmee bereik je alles wat je maar wilt. Ben trots op jou!

Hann, je bent de beste! Ik ben heel trots op je hoe je elke keer weer je plek weet te veroveren in een bedrijf in een nieuwe omgeving! Dankjewel dat je mijn paranimf wilt zijn en kom maar gauw terug naar Nederland! Love you, two!

Papa en mama. Dit boek is voor jullie. Jullie zijn de beste ouders die iemand zich maar kan wensen. Het grote vertrouwen dat jullie altijd in mij hebben, samen met jullie onvoorwaardelijke steun, geeft mij de energie en de zin om dingen te doen waarvan ik niet voor ogen had dat ik ze kon. Ik ben enorm trots dat ik jullie dochter ben en hou heel veel van jullie!
Liefste Rolf. Na exact 1 jaar promoveren leerden wij elkaar kennen. En sindsdien zijn we onafscheidelijk! Mijn liefde voor jou is niet onder woorden te brengen. Dankjewel dat jij er altijd voor me bent, zo goed voor me zorgt en zoveel van mij houdt. Jij bent de basis die ik nodig heb. Met jou is elke dag een feestje en daar gaan er nog héél veel van komen. Ik kijk heel erg uit naar mijn leven samen met jou.