Pivotal Study of the NanoKnife System for Ablation of Prostate Tissue in an Intermediate-Risk Patient Population

Protocol Number: 2021-ONC-01

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Pivotal Study of the NanoKnife System for Ablation of Prostate Tissue in an Intermediate-Risk Patient Population

2021-ONC-01
IDE No. G210154
January 20, 2023
Version 4.0

AngioDynamics, Inc.
14 Plaza Drive
Latham, NY 12110

Confidentiality Statement:

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Good Clinical Practice Statement:

This study will be conducted in accordance with Good Clinical Practices (GCP and ISO 14155:2011) and applicable regulatory requirements, including the archiving of essential documents.

PROTOCOL APPROVAL

Pivotal Study of the NanoKnife System for Ablation of Prostate Tissue in an Intermediate-Risk Patient Population

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1/26/2023
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Jonathan Coleman, MD – Principal Study Investigator, Urologist, Memorial Sloan Kettering

1/26/2023
Date

INVESTIGATOR STATEMENT

Pivotal Study of the NanoKnife System for Ablation of Prostate Tissue in an Intermediate-Risk Patient Population

Protocol Number: 2021-ONC-01
Revision Date: January 20, 2023
Version Number: 4.0
IDE Number: G210154

I agree to conduct the above referenced clinical study protocol in accordance with the design and specific provisions as designated in this protocol. Modifications to the study protocol are acceptable only in the form of a protocol amendment. I agree to await Institutional Review Board and AngioDynamics approval for the protocol, informed consent and documentation to be presented to patients before initiating the study, to obtain informed consent from patients prior to their enrollment into the study, to collect and record data as required by this protocol and case report forms, to report non serious and serious adverse events that may occur for any patient participating in this study under my care, to report product complaints for any of the devices utilized in this protocol, and to maintain study related documentation (regulatory documentation) for the period of time required. I agree to inform AngioDynamics if I have been involved in an investigation or other research that was terminated, and if so, an explanation of the circumstances that led to the termination. I have read and understand the contents of this protocol. I agree to follow and abide by the requirements set forth in this document.

I understand the information in this document contains trade secrets and commercial information that are privileged or confidential and may not be disclosed unless such disclosure is required by applicable law or regulations. In any event, persons whom the information is disclosed must be informed that the information is privileged or confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future information supplied to me, which is indicated as privileged or confidential.

_____
Principle Site Investigator Name (print)

_____ _____
Principle Site Investigator Signature Date

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PROTOCOL SUMMARY

Protocol #:	2021-ONC-01
Protocol Title:	Pivotal Study of the NanoKnife System for the Ablation of Prostate Tissue in an Intermediate-Risk Patient Population
Purpose:	To evaluate the safety and effectiveness of the NanoKnife System to ablate prostate tissue when used in an intermediate-risk patient population.
Design:	This study will be a prospective, non-randomized pivotal study in 118 subjects at up to 20 clinical sites in the United States.
Enrollment:	This study will treat 118 subjects and follow them for the primary and secondary endpoint analyses at 12 months.
Study Objectives:	**Primary Objectives:** 1. To determine the NanoKnife System's ablation effectiveness by measuring the negative in-field biopsy rate at 12 months. 2. To determine the NanoKnife System's procedural and post-procedural safety profile by evaluating adverse event incidence, type, and severity through 12 months. **Secondary Objectives:** 1. To evaluate urinary and erectile function after NanoKnife System treatment using validated subject questionnaires (UCLA-EPIC, IPSS, IPSS-QOL, IIEF-15). 2. To determine post-NanoKnife System treatment prostate-specific antigen (PSA) kinetics, including time to PSA nadir and post-nadir PSA stability. 3. To determine the change in prostate volume by comparison of prostate volume measured on multiparametric MRI (mpMRI) pre-treatment and at 12 months post-treatment. 4. To determine the effectiveness of therapy by assessing the need for secondary or adjuvant treatment following therapy. 5. To determine health-related quality of life (HRQoL) levels after treatment with the NanoKnife System using a validated subject questionnaire (EQ-5D).
Eligibility Criteria:	**Inclusion Criteria** A subject is required to fulfill all the following criteria to be included in the study: 1. Is greater than 50 years of age 2. Has at least a 10-year life expectancy 3. Has histologically confirmed organ-confined prostate cancer, clinical stage ≤ T2c

	4. Has a PSA ≤ 15 ng/mL or PSA density < 0.15 ng/mL^2 if PSA is > 15 ng/mL 5. Has Gleason score 3+4 or 4+3 6. Has no evidence of extraprostatic extension by mpMRI 7. Has no evidence of seminal vesicle invasion by mpMRI, and if suspected, confirmed by biopsy 8. Physician is able to visualize prostate gland adequately on transrectal ultrasound imaging during qualifying biopsy 9. Has a transperineal or transrectal targeted prostate biopsy of lesion, plus 10-14 core systematic biopsy to include adequate sampling of the peripheral zone correlating with an intermediate risk lesion[1] in the area of the MR-visible lesion 10. A visible lesion on mpMRI that is accessible to Irreversible Electroporation (IRE) treatment (Note: prostate cancer detected via systematic standard biopsy outside of the adjacent sextant location of the MRI visible lesion will meet entry criterion provided the positive core is Gleason 6; has fewer than 3 prostate biopsy fragments/cores positive and ≤50% cancer in each of those fragments/cores on standard biopsy) 11. Is willing and able to sign a written informed consent form and in the judgment of the physician, the study is in the best interest of the subject 12. Understands and accepts the obligation and is logistically able to present for all scheduled follow-up visits **Exclusion Criteria** A subject will be excluded from participation in the study if they meet any of the following criteria: 1. Has known hypersensitivity to pancuronium bromide, atricurium or cisatricurium 2. Is unfit for anesthesia or has a contraindication for agents listed for paralysis 3. Has an active urinary tract infection (UTI) 4. Has a history of bladder neck contracture 5. Is interested in future fertility 6. Has a history (within 3 years) of inflammatory bowel disease 7. Has a concurrent major debilitating illness 8. Had active treatment for a malignancy within 3 years, including malignant melanoma, except for prostate cancer or other types of skin cancer (Note: subjects with untreated active concomitant cancers are excluded, only subjects deemed to be in remission by their cancer care provider for at least three years are eligible) 9. Has any active implanted electronic device (e.g., pacemaker)

[1] An intermediate risk lesion is defined as Gleason score 3+4 or 4+3, PSA < 15 ng/mL or PSA density < 0.15 ng/mL^2 if PSA is > 15 ng/mL, and ≤ clinical stage T2c.

	10. Is unable or unwilling to catheterize 11. Has had any prior or current prostate cancer therapy, including: a) Biologic therapy for prostate cancer b) Chemotherapy for prostate cancer c) Hormonal therapy for prostate cancer within three months of procedure d) Radiotherapy for prostate cancer e) Surgery for prostate cancer 12. Has had prior transurethral prostatectomy (TURP), stricture surgery, urethral stent or prostatic implants 13. Has had prior major rectal surgery (except hemorrhoids) 14. Is unfit for pelvic MRI scanning (e.g., severe claustrophobia, permanent cardiac pacemaker, metallic implants that are likely to contribute significant image artifacts, allergy or contraindication to gadolinium (to enhance MRI)) 15. Is actively bleeding, has a bleeding disorder, or is unable to interrupt blood thinning medications as clinically indicated per pre-operative best practices 16. Is a member of a vulnerable population, such as cognitively impaired or incarcerated, that could expose them to undue influence, coercion, or inability to obtain informed consent 17. In the opinion of the treating physician, has a contraindication listed in the current NanoKnife System User Manual (section 2.3)
Primary Safety Endpoint:	Incidence of adverse events by type and CTCAE v5.0 severity through 12 months.
Primary Effectiveness Endpoint:	Rate of negative in-field biopsy at 12 months.
Secondary Endpoints:	• Rate of negative in-field biopsy at 12 months as defined by the Delphi consensus criterion of absence of clinically significant disease (≤ 3 mm of Gleason ≤ 6 disease in any biopsy core is insignificant)[1] • Assessment of urinary function by comparison of pre- and post-operative UCLA Expanded Prostate Cancer Index Composite[2] (UCLA-EPIC) Urinary Domain and International Prostate Symptom Scores[3] (IPSS) and IPSS Quality of Life (IPSS-QoL) scores. • Assessment of erectile function by comparison of pre- and post-operative 15-Item International Index of Erectile Function[4] (IIEF-15) potency scores. • Effectiveness of therapy by measurement of prostate-specific antigen (PSA) kinetics including time to PSA nadir. • Assessment of changes in prostate volume by comparison of pre-treatment and 12-month prostate volume measured via mpMRI. • Assessment of ablation effectiveness by evaluation of prostate tissue by mpMRI at 3 months post-treatment and at 12 months post-treatment. • Assessment of need for secondary or adjuvant treatment following treatment with the NanoKnife System.

	• Evaluation of subject reported pre- and post-operative Quality of Life (QoL) using the 5-dimension scale EuroQol[5] (EQ-5D®).
Exploratory Endpoint:	• Rate of progression, re-intervention, and adverse events collected as part of standard of care through five years.
Study Procedure:	Prospective subjects who sign a written informed consent form will be enrolled in the study. Study eligibility will be confirmed at the Screening visit. Qualifying transperineal or transrectal prostate biopsy must have been performed no more than 180 days prior to the time of enrollment. If they continue to meet enrollment criteria, they will be scheduled for treatment and after undergoing standard bowel preparation, the NanoKnife System procedure will be carried out under general anesthesia and utilizing transrectal ultrasound (US) guidance.
Study Schedule:	Subjects will undergo treatment with the NanoKnife System as well as follow-up visits at 1, 3, 6, 9, and 12 months post-procedure. Transrectal US and transperineal or transrectal biopsy will be completed at the 12-month follow up visit. Post-treatment imaging will be completed using mpMRI scan at 3 months post-procedure and at 12 months post-procedure.
Statistical Analysis:	Analysis of all efficacy endpoints will be performed on both the intent-to-treat (ITT) population and the per-protocol (PP) population, with the ITT population serving as the primary analysis. Analysis of all safety endpoints will be performed on the ITT population. • Intent-to-Treat Population (ITT): This population includes all subjects who were enrolled and treated with the NanoKnife System • Per Protocol Population (PP): This population includes all subjects who were enrolled and treated with the NanoKnife System, and who had no major protocol deviations

1. INTRODUCTION

Prostate cancer is a malignant tumor that arises in the prostate gland. As with any cancer, if it is advanced or left untreated in early stages, it can eventually spread throughout the blood and lymph fluid to other organs.

It is estimated that by age 70, about 65% of men exhibit some evidence of prostate cancer. This type of cancer occurs almost exclusively in men over age 40 and most often after age 50. The National Cancer Institute estimates there will be 248,530 new cases and 34,130 deaths attributed to prostate cancer in the United States in 2021.[6]

In July 2020, FDA released a guidance document, *Clinical Investigations for Prostate Tissue Ablation Devices*, which provides clinical testing recommendations for manufacturers seeking a general indication for prostate tissue ablation. In this protocol, references to prostate cancer are only intended to describe typical prostate tissue ablation scenarios. This study is being conducted to support a prostate tissue indication, not a prostate cancer indication.

This protocol will enroll intermediate-risk prostate cancer patient (Gleason 3+4 or Gleason 4+3). NCCN guidelines state that low-risk (Gleason 3+3) cancer is considered “insignificant” and rarely leads to metastatic disease or patient mortality.[7] This patient population is best suited for active surveillance and monitoring. Intermediate-risk disease (Gleason score of 3+4 and 4+3) is considered the ideal patient group worthy for investigation for focal therapy.[8]

Focal therapy is seen as a middle ground treatment option to either delay or disqualify the need for definitive whole gland treatment, which is very effective at cancer control but exposes the patient to severe quality of life alterations. This study aims to investigate the safety and efficacy of Irreversible Electroporation in the ablation of prostate tissue.

2. STUDY DEVICE OVERVIEW

The NanoKnife System is a reusable, non-sterile device that operates outside of the sterile field and consists of a Touchscreen LCD Display, Console and Keyboard, Power Unit and Power Cord, and a Double Footswitch. The Generator is connected to sterile, single-use, disposable NanoKnife Electrode Probes, which are invasive devices that are used in the sterile field. The NanoKnife System has six probe outputs which allow users to connect up to six NanoKnife Electrode Probes at one time, however; only one pair of NanoKnife Electrode Probes can be operated at a time. The Electrode Probe Spacer is a sterile, single-use optional accessory to aid in spacing the probes at a fixed distance and aid in keeping the probes parallel.

The NanoKnife System is a software-controlled low-energy direct-current (LEDC) generator which surgically ablates soft tissue. With the NanoKnife System, a voltage is applied between pairs of probes in a series of pulses. The waveform of the voltage is adjustable as determined by clinician-chosen parameters. These parameters include volts/cm, pulse length, number of pulses to be delivered between electrode pairs, the distance between probes, and the timing mode (90PPM or ECG synchronization). Up to six probes may be placed in an array within the tissue. The probes of the array are matched as pairs by the system. When probes are activated via a foot-pedal, the scheduled voltage is delivered to tissue between subsequent pairs of probes. Soft tissue between the probes is ablated.

The NanoKnife System's Indications for Use is: *The NanoKnife System with six outputs is indicated for the surgical ablation of soft tissue.*

During the procedure, the electrical pulses between probe pairs produce an electric field which induces electroporation of cells within the targeted ablation area. Electroporation is a technique in which an electrical field is applied to cells in order to increase the permeability of the cell membranes through the formation of nanoscale defects (openings called nanopores) in the lipid bilayer.

After delivering a sufficient number of high voltage pulses, the cells surrounding and between the electrodes will be irreversibly damaged, changing the permeability of the membrane. The cell membrane damage leads to apoptosis as the cells lose the ability to maintain homeostasis. This mechanism which causes permanent cell damage is referred to as Irreversible Electroporation (IRE).

IRE of the prostate is typically performed with the subject in the lithotomy position, with 2-6 monopolar probes placed through the perineum using a brachytherapy grid and ultrasound or CT guidance. IRE supplies the targeted tissue with high voltage (2-3 kV) direct current pulses lasting up to 100 microseconds through the electrode probes.

The performance specifications for the NanoKnife System are listed in the table below.

Table 2-1: The NanoKnife System Specifications

Component	Description
Number of Probe Outputs	1 – 6*

Component	Description
Number of Pulses**	10 to 100
Pulse Amplitude	500 to 3000 V
Pulse Length	20 - 100 µ s
Pulse Interval, Un-sync	90 PPM, 670 ms/3.5 s every 10th pulse
Pulse Interval, Sync	ECG, interval varies depending on heart rate
Maximum Energy per Pulse (Nominal)	15 J
Energy Storage***	100 µ F minimum
Pulse Amplitude Precision	±5%
Pulse Length Precision	±2 µ s or 2% (whichever is larger)
Maximum Current	50 A

* A minimum of 2 probes are required
** Number of pulses for each pair of electrodes.
*** Between recharges

The NanoKnife System User Manual is available in **Appendix D**.

2.1 Regulatory Status

The NanoKnife System has received marketing clearance from the US Food and Drug Administration via 510(k) K183385 for the surgical ablation of soft tissue. For the purposes of this protocol, the NanoKnife System is considered a significant risk investigational device.

The investigation described within the protocol will be conducted in strict accordance with the rules and regulations of the United States Food and Drug Administration, e.g., 21 CFR 50, 56, 812.

This investigation will be conducted under an FDA approved Investigational Device Exemption (G210154). The NanoKnife System is a CE marked medical device in Europe. NanoKnife System has received ISO 13485:2003 & EN ISO 13485:2012 Quality Management System certificate MD 622301 and EC Certificate CE 559984 (Directive 93/42/EEC on Medical Devices, Annex II Excluding Section 4), which allows AngioDynamics to apply the CE mark to the NanoKnife System. These certificates indicate that the company's quality system and NanoKnife System design technical documentation have been reviewed and approved by the European Notified Body.

3. STUDY PURPOSE AND DESIGN

3.1 Purpose

The purpose of this study is to evaluate the safety and effectiveness of the NanoKnife System to ablate prostate tissue when used in an intermediate-risk patient population.

3.2 Design

This study is designed to be a prospective, non-randomized pivotal study in 118 subjects treated at up to 20 clinical sites in the United States.

This study will involve 118 subjects who meet the intermediate-risk criteria defined by this protocol. The biopsy and imaging techniques that we will adopt within this trial are multiparametric MRI (mpMRI) and transperineal or transrectal prostate biopsy (fusion targeted biopsy of lesion plus 10-14 core systematic biopsies to include adequate sampling of peripheral zone). The subjects' prostate lesions, the locations of which will be determined by ultrasound-guided transperineal or transrectal prostate biopsy, will be targeted for treatment with the NanoKnife System. The primary objective of this the study will be to evaluate the NanoKnife System's ablation effectiveness by measuring the negative in-field biopsy rate at 12 months, and the procedural and post-treatment safety of the NanoKnife System via incidence and severity of adverse events through 12 months. The secondary objective of this study is to evaluate urinary and erectile function after NanoKnife System treatment using validated subject questionnaires, and to evaluate pre- and post-treatment changes in PSA and prostate volume. Other secondary outcomes include the effectiveness of the NanoKnife System by assessing the need for secondary or adjuvant treatment and health-related quality of life evaluated using validated subject questionnaires.

This group of subjects will be followed for safety and efficacy for 12 months. Safety will be assessed via incidence and severity of adverse events and evaluation of the treatment effect on urologic function and quality of life. Local efficacy will be characterized via negative biopsy, post-treatment prostate-specific antigen (PSA) kinetics including time to PSA nadir and post-nadir PSA stability, and changes in prostate volume.

3.2.1 Identifying the Disease by Multiparametric MRI

Multiparametric MRI will be the non-invasive investigation on which the presence of intermediate-risk[2] lesions amenable to focal ablation will be identified. This pre-treatment imaging will already have been performed, prior to being enrolled in the study during the initial screening visit.

Upon screening mpMRI, no evidence of extraprostatic extension and no evidence of seminal vesicle invasion is required.

Extraprostatic extension (EPE) will be evaluated using the 5-point Likert scale from modified PI-RADS criteria ESUR[9]:

[2] An intermediate risk lesion is defined as Gleason score 3+4 or 4+3, PSA < 15 ng/mL or PSA density < 0.15 ng/mL[2] if PSA is > 15 ng/mL, and ≤ clinical stage T2c.

1 = EPE absent (Normal tissue can be visualized between intact prostate capsule and tumor),
2 = EPE probably not present (Tumor abuts prostate capsule),
3 = Equivocal for EPE (Tumor abuts and causes irregularity on prostate capsule),
4 = EPE probably present (Tumor bulges, deforms, and obscures the prostate capsule),
5 = EPE absolutely present (Gross and measurable tumor is identified).

Accordingly, any scale of 1, 2, or 3 lesion will be negative for extraprostatic extension, while any scale 4 or 5 lesion will be positive for extraprostatic extension.

Seminal vesicle invasion will be evaluated using the following 4-point description of features from modified PI-RADS criteria that should lead to further investigation of the seminal vesicle[10]:

1 = Seminal vesicles Expansion
2 = Low T2 signal
3 = Filling in of angle
4 = Enhancement and impeded diffusion

If there is possible suspicion for seminal vesicle invasion on the mpMRI (score of 1-4), negative targeted biopsy should be performed as clinically indicated for a subject to be considered eligible for inclusion in the study.

Pre-Operative 3-Month, and 12-Month mpMRI Protocol:

Pre-operative and all post-NanoKnife System imaging will be performed utilizing the Prostate Imaging – Reporting and Data System Version 2.1 (PI-RADS version 2.1).

See **Appendix A – Standard Operating Procedure: 1. mpMRI Protocol** for imaging protocol details.

3.2.2 Disease Localization: Transperineal or Transrectal Prostate Biopsies

The initial biopsy will already have been performed prior to enrollment into the study, demonstrating eligibility for inclusion. The process by which the specified distribution of lesions will be verified will be with transperineal or transrectal fusion targeted biopsy of lesion plus 10-14 core systematic biopsy to include adequate sampling of peripheral zone. Three-dimensional data on the location and specific grade for each lesion will be available and focal ablation planning will be based on this information. PSA for inclusion in the study is to have been performed prior to the initial biopsy, or it may be performed after biopsy as long as it is taken greater than 30 days after biopsy.

See **Appendix A – Standard Operating Procedure: 2. Transperineal and Transrectal Prostate Biopsies Protocol** for full details.

3.3 Objectives

3.3.1 Primary Objectives

- To determine the NanoKnife System's ablation effectiveness by measuring the negative in-field biopsy rate at 12 months.
- To determine the NanoKnife System's procedural and post-procedural safety profile by evaluating adverse event incidence, type, and severity through 12 months.

3.3.2 Secondary Objectives

- To evaluate urinary and erectile function after NanoKnife System treatment using validated subject questionnaires (UCLA-EPIC, IPSS, IPSS-QOL, IIEF-15).
- To determine post-NanoKnife System treatment prostate-specific antigen (PSA) kinetics, including time to PSA nadir and post-nadir PSA stability.
- To determine the change in prostate volume by comparison of prostate volume measured on multiparametric MRI (mpMRI) pre-treatment and at 12 months post-treatment.
- To determine the effectiveness of therapy by assessing the need for secondary or adjuvant treatment following therapy.
- To determine health-related quality of life (HRQoL) levels after treatment with the NanoKnife System using a validated subject questionnaire (EQ-5D).

3.4 Hypotheses

The primary efficacy analysis will be the rate of subjects with a negative in-field biopsy at 12 months. The rate of negative in-field biopsies will be compared to a performance goal of 0.52 using a one-sample non-inferiority test for proportions.

The null and alternative hypotheses for negative biopsy rate at 12 months are as follows:

H_0: NanoKnife System ≤ (0.61 – margin of 0.09)
H_1: NanoKnife System > (0.61 – margin of 0.09)

For safety, the study will be able to detect at least one medically significant adverse event of any type with over 80% accuracy if the true event rate is at least 1.6%.

3.5 Endpoints and Procedures

3.5.1 Primary Endpoint

The primary treatment outcomes that will be evaluated are:

- Rate of negative in-field biopsy at 12 months – determine the rate of subjects obtaining a negative in-field biopsy on follow-up transperineal or transrectal biopsy at 12 months.
- Incidence of adverse events by type and CTCAE v5.0 severity through 12 months – determine incidence, type, and severity during the NanoKnife System procedure and through follow-up.

3.5.2 Secondary Endpoints

The secondary outcomes that will be evaluated are:

- Rate of negative in-field biopsy at 12 months as defined by the Delphi consensus criterion of absence of clinically significant disease (≤ 3 mm of Gleason ≤ 6 disease in any biopsy core is insignificant)[1]
- Assessment of urinary function by comparison of pre- and post-operative UCLA Expanded Prostate Cancer Index Composite (UCLA-EPIC) Urinary Domain[2] and International Prostate Symptom Scores (IPSS)[3] and IPSS Quality of Life (IPSS-QoL) scores.
- Assessment of erectile function by comparison of pre- and post-operative 15-Item International Index of Erectile Function (IIEF-15)[4] potency scores.
- Effectiveness of therapy by measurement of prostate-specific antigen (PSA) kinetics including time to PSA nadir.
- Assessment of changes in prostate volume by comparison of pre-treatment and 12-month prostate volume measured via mpMRI.
- Assessment of ablation effectiveness by evaluation of prostate tissue by mpMRI at 3 months post-treatment and at 12 months post-treatment.
- Assessment of need for secondary or adjuvant treatment following treatment with the NanoKnife System.
- Evaluation of subject reported pre- and post-operative Quality of Life (QoL) using the 5-dimension scale EuroQol (EQ-5D®).[5]

3.5.3 Exploratory Endpoint

Subjects will be continued to be followed up as Standard of Care and medical records will be made available for up to five years, in order to assess exploratory endpoints:

- Rate of progression, re-intervention, and adverse events collected as standard of care through five years.

4. SUBJECT ELIGIBILITY AND STUDY VISITS

4.1 Pre-Screening

Subjects will be pre-screened for eligibility as described below:

Medical records of the investigator's subjects will be reviewed by the investigator to identify persons who may be eligible for this study. Subjects who are determined to potentially be eligible will be contacted by the investigator and offered participation in the study. During this initial contact the investigator will explain the study, the procedure and any risks or benefits anticipated, and determine if the subject would be interested in participating. If a subject expresses interest in participating, he will be determined a pre-screening success and will be scheduled to meet the investigator for a more detailed explanation of the study and for the Informed Consent process.

The subject's initials, date of screening, whether the subject was a screening success or failure, and reason for failure (if a failure) or subject ID (if a success) will be entered on the Screening Log.

4.2 Informed Consent

Informed consent will be obtained under the conditions set forth in 21 CFR Part 50: a) the subject shall have sufficient opportunity to consider participation in the study, b) informed consent shall be obtained without coercion or undue influence, c) informed consent shall be written in the native language of the subject and administered by approved personnel who speak the native language of the subject, d) a subject cannot be led to believe that they are waiving their rights as a subject or the liability of the sponsor or investigator.

Informed Consent Forms will be signed and dated by the subject prior to the completion of any study-specific procedures. Consent will be administered by the investigator or other person authorized by the Institutional Review Board (IRB) such as a study coordinator or a translator. A copy of the Informed Consent Form once signed and dated will be provided to the subject.

Subjects will be considered enrolled in the study after consent has been obtained.

Informed Consent Forms will follow the format of elements presented in 21 CFR Part 50.25. See **Appendix C – Informed Consent Form.**

4.3 Eligibility Criteria

Subjects will be assessed for inclusion into this study using the criteria identified below. Subjects must meet all inclusion criteria and not meet any exclusion criteria in order to qualify for the study.

4.3.1 Inclusion Criteria

A subject is required to fulfill all of the following criteria to be included in the study:

1. Is greater than 50 years of age

2. Has at least a 10-year life expectancy
3. Has histologically confirmed organ-confined prostate cancer, clinical stage ≤ T2c
4. Has a PSA ≤ 15 ng/mL or PSA density < 0.15 ng/mL^2 if PSA is > 15 ng/mL
5. Has Gleason score 3+4 or 4+3
6. Has no evidence of extraprostatic extension by mpMRI
7. Has no evidence of seminal vesicle invasion by mpMRI, and if suspected, confirmed by biopsy
8. Physician is able to visualize prostate gland adequately on transrectal ultrasound imaging during qualifying biopsy
9. Has a transperineal or transrectal targeted prostate biopsy of lesion, plus 10-14 core systematic biopsy to include adequate sampling of the peripheral zone correlating with an intermediate risk lesion[3] in the area of the MR-visible lesion
10. A visible lesion on mpMRI that is accessible to Irreversible Electroporation (IRE) treatment (Note: prostate cancer detected via systematic standard biopsy outside of the adjacent sextant location of the MRI visible lesion will meet entry criterion provided the positive core is Gleason 6; has fewer than 3 prostate biopsy fragments/cores positive and ≤50% cancer in each of those fragments/cores on standard biopsy)
11. Is willing and able to sign a written informed consent and in the judgment of the physician, the study is in the best interest of the subject
12. Understands and accepts the obligation and is logistically able to present for all scheduled follow-up visits

4.3.2 Exclusion Criteria

A subject will be excluded from the study if they meet any of the following criteria:

1. Has known hypersensitivity to pancuronium bromide, atricurium or cisatricurium
2. Is unfit for anesthesia or has a contraindication for agents listed for paralysis
3. Has an active urinary tract infection (UTI)
4. Has a history of bladder neck contracture
5. Is interested in future fertility
6. Has a history (within 3 years) of inflammatory bowel disease
7. Has a concurrent major debilitating illness
8. Had active treatment for a malignancy within 3 years, including malignant melanoma, except for prostate cancer or other types of skin cancer
9. Has any active implanted electronic device (e.g., pacemaker)
10. Is unable or unwilling to catheterize
11. Has had any prior or current prostate cancer therapy, including:
    a) Biologic therapy for prostate cancer
    b) Chemotherapy for prostate cancer
    c) Hormonal therapy for prostate cancer within three months of procedure
    d) Radiotherapy for prostate cancer
    e) Surgery for prostate cancer
12. Has had prior transurethral prostatectomy (TURP), stricture surgery, urethral stent or prostatic implants

[3] An intermediate risk lesion is defined as Gleason score 3+4 or 4+3, PSA < 15 ng/mL or PSA density < 0.15 ng/mL^2 if PSA is > 15 ng/mL, and ≤ clinical stage T2c.

13. Has had prior major rectal surgery (except hemorrhoids)
14. Is unfit for pelvic MRI scanning (e.g., severe claustrophobia, permanent cardiac pacemaker, metallic implants that are likely to contribute significant image artifacts, allergy or contraindication to gadolinium (to enhance MRI))
15. Is actively bleeding, has a bleeding disorder, or is unable to interrupt blood thinning medications as clinically indicated per pre-operative best practices
16. Is a member of a vulnerable population, such as cognitively impaired or incarcerated, that could expose them to undue influence, coercion, or inability to obtain informed consent
17. In the opinion of the treating physician, has a contraindication listed in the current NanoKnife System User Manual (section 2.3)

The location and grade of the subjects' prostate lesion will be documented with the fusion targeted biopsy of the MRI-visible lesion plus 10-14 core systematic biopsy to include adequate sampling of peripheral zone. See **Appendix A – Standard Operating Procedure: 2. Transperineal and Transrectal Prostate Biopsies Protocol** for full details.

All laboratory evaluations will be conducted by each investigational site's local licensed clinical laboratory.

4.4 Baseline

Study subjects who have completed all screening assessments and remain eligible for inclusion in the study will be scheduled for a Baseline Assessment visit. Screening and Baseline visits may be combined. At the time the subject presents for baseline, an assessment will be made to determine whether the subject still meets the Inclusion/Exclusion criteria.

The following data will be collected at the Baseline Visit, which must be completed within 30 days prior to treatment with the NanoKnife System:

4.4.1 Physical Examination and Vital Assessments

All subjects will have a full standard physical examination including height, weight, temperature, blood pressure, respiration rate and pulse.

4.4.2 Laboratory Tests

Blood samples will be drawn for complete blood count (CBC), prothrombin time (PT), and partial thromboplastin time (PTT).

Urine samples will be obtained if there is any question of an active urinary tract infection.

4.4.3 Quality of Life (QoL) Assessment

The following tools will be used to assess subjects' pre-operative quality of life:

- Urinary Function Score: The UCLA Expanded Prostate Cancer Index Composite (UCLA-EPIC)[2] Urinary domain and International Prostate Symptom Score
- (IPSS) and IPSS Quality of Life (IPSS-QoL)[3] scores to assess preoperative urinary symptoms will be obtained. See **Appendix E – Subject Questionnaires – 1. Urinary Function Score** for more details on Questionnaire and Scoring instructions.
- Erectile Function Score: The International Index of Erectile Function[4] (IIEF-15) potency score to assess preoperative erectile function will be obtained. See **Appendix E – Subject Questionnaires – 2. Erectile Function Score** section for more details on Questionnaire and Scoring instructions.
- Overall Quality of Life: The 5-dimension scale EuroQol[5] (EQ-5D®) to assess preoperative quality of life will be obtained. See **Appendix E – Subject Questionnaires – 3. Quality of Life (QoL) Score** section for more details on Questionnaire and Scoring instructions.

4.5 Procedure Preparation

Study subjects will be given pre-procedure instructions which will detail their food, liquid and medication intake for the day before and day of treatment.

Study subjects will be admitted to the hospital for the procedure. On the day of the scheduled treatment subjects will be requested to present promptly at the specified time in order to complete all pre-treatment preparations.

Subjects will have an abbreviated physical exam, including vital assessments and weight.

Study subjects will need to undergo standard bowel preparation with a phosphate enema as required for clear visualization of the prostate on ultrasound.

4.6 Study Procedure

Subjects will be placed in the dorsal lithotomy position under sterile technique. The NanoKnife System procedure will be carried out under general anesthesia. A Foley catheter will be placed to aid in draining the bladder during treatment.

Prior to treatment with the NanoKnife System, subjects will receive Ciprofloxacin 400 mg or another antibiotic of choice selected by the treating physician per institutional guidelines via intravenous infusion to reduce the chance of infection. Subjects will be dosed according to the Dosage Guidelines in the product package insert of the chosen antibiotic. Institutional guidelines should also be followed regarding pre-operative heparin.

The area of the prostate that was positive for cancer-bearing tissue based on the mpMRI and transperineal or transrectal prostate biopsy will be targeted for focal ablation via the NanoKnife System. Only the prostate cancer-bearing tissue will be targeted for ablation, however, a treatment margin of greater than or equal to 5 mm around the Gleason 3+4 or 4+3 (intermediate-risk) lesion should be included. Note: if on biopsy, the subject was found to have a lesion in the contralateral hemisphere of the prostate that is Gleason 6 and comprises no more than 6 mm linear extent of prostate-bearing tissue in a single core, this lesion should not be treated.

An MRI/transrectal ultrasound (TRUS) fusion device or standard TRUS probe may be placed in the rectum to visualize the prostate in both sagittal and axial views. The ultrasound grid which was used during the mapping biopsy will be oriented using anatomical landmarks and used to identify the location of the positive biopsy cores. The NanoKnife Single Electrode Probes will be surgically inserted into the prostate through the perineum using MRI/TRUS fusion guidance and the ultrasound grid for guidance. The location of the probes will be documented via ultrasound imaging and notation in the Case Report Form (CRF).

Further details of the further details of the procedures listed below can be found in the Clinical Study Guide provided.

4.6.1 Measure the Prostate

Volumetric analysis of the prostate will be completed during the pre-operative mpMRI as well as the 3-month and 12-month post-operative mpMRIs. The mpMRI images and volumetric analyses will be saved in the study file.

4.6.2 Set the Insulation Length for the Probes

Using the slider on the handle of the probe, set the slider to the determined electrode tip exposure for the planned ablation zone as determined by transrectal ultrasound. A probe exposure of 1.0-2.0 cm is recommended to avoid overcurrent conditions. The ablation extends 5 mm beyond the exposed electrode tip and 5 mm proximal to the exposed electrode tip. Choose the electrode tip exposure length to provide the necessary coverage of the intended ablation zone. The maximum electrode tip exposure should not exceed 2.0 cm. More than one ablation may be required to treat the entire length of the prostate. For example, if the prostate measures 4 cm in length, the maximum probe exposure (2.0 cm) will ablate a 3.0 cm long area of prostate. Then the electrode must be repositioned to ablate the remaining length of untreated prostate tissue if desired.

4.6.3 Deliver Neuromuscular Blockade

After placement of the NanoKnife probes in the prostate and immediately prior to NanoKnife treatment, a nondepolarizing neuromuscular blocking agent will be administered to reduce skeletal muscle contraction which is associated with the use of the NanoKnife System. It is important that the subject is deeply paralyzed during energy delivery (zero twitches from a train of four).

4.6.4 Prepare to Apply Treatment

An MRI/TRUS fusion device which integrates mpMRI images with real-time transrectal ultrasound images can be used to aid in the placement of electrodes and determine the actual distance between probes. For full details on the MRI/TRUS fusion protocol, see **Appendix A - Standard Operating Procedure: 3. Optional MRI/TRUS Fusion Image Registration for NanoKnife Treatment Planning.**

The physician will input the inter-probe distances by updating the probe icon locations in the probe placement grid (part of the NanoKnife software). Each probe icon is positioned around the probe placement grid until the inter-probe distances are accurate for each probe pair. A calculator tool,

which allows the physician to input the inter-probe distances, is available and can be used to assist the physician in positioning the probe icons in the probe placement grid.

The treatment parameters will be 1500 V for probes placed 10 mm apart. Voltage is calculated by multiplying the inter-probe distance of any given probe pair (in centimeters) by the V/cm setting in the software. For example, 1.0 cm × 1500 V/cm = 1500 V.

By default, the software uses 1500 V/cm as the initial V/cm setting for all probe pairs. The physician can modify the V/cm setting for any probe pair listed within the parameters table via the software. For prostate ablation, it is advisable to use the 1500 V/cm setting for inter-probe spacing of 1.0 cm and greater (to a maximum of 2.0 cm).

Probe pairs with inter-probe spacing greater than 2.0 cm should either be removed from the parameters table or the probes should be positioned closer together so that the inter-probe spacing is 2.0 cm or less. Probe pairs with inter-probe spacing of less than 1.0 cm should be removed from the parameters table or repositioned to an inter-probe distance of 1.0 cm or greater if possible considering anatomy and intended treatment zone.

4.6.5 Treatment Parameters

All treatments will utilize a total of 90 pulses with each pulse having a duration of 90 µsec. The pulses should be delivered asynchronously using the 90 PPM (pulses per minute) setting.

The NanoKnife System will prompt the user to enter the treatment parameters for each probe pairing. The treatment will be delivered between every electrode pair in a bipolar manner until all the permutations for the probe array are covered.

4.6.6 Full Voltage Test Pulses

In order to ensure that current draw is within the zone of irreversible electroporation (20 to 35 amps), 10 full voltage test pulses should be delivered. After the desired baseline amperage is obtained the remaining 80 pulses will be delivered for a total of 90 pulses within desired treatment parameters.

Prior to advancing to the Pulse Generation Screen, review the following items:

Table 4-2. Pre-treatment Checklist

1.	Verify probe icon numbers correlate to actual probe numbers from the measurements taken
2.	Verify all intended probe pairs are present and unintended probe pairs have been removed
3.	Verify all the pulse lengths are 90 µsec
4.	Verify all probe pairs are set to deliver 10 pulses (Full-Voltage Test Pulse Sequence) or 80 pulses (Treatment Sequence)
5.	Verify all the inter-probe distances are between 1.0 cm and 2.0 cm
6.	Ensure each of the probe's electrode exposures have been entered correctly
7.	Confirm with anesthesia team that paralytic is on board and the subject displays "0 twitches"

At this point the user can initiate the treatment. The previously programmed pulse sequences will then be applied to each pair of probes.

Review the results graphs immediately upon the completion of the Full-Voltage Test Pulse Sequence. If all the Full-Voltage Test Pulse amplitudes are between 20 and 35 amps, the full-voltage test pulse sequence has been completed successfully. Amplitudes below 20 amps can adversely affect the accuracy of the IRE treatment zone boundaries, while amplitudes greater than 35 amps can increase the incidence of over-current conditions during treatment and increase targeted tissue temperature.

4.6.7 NanoKnife Treatment

After successfully completing the Full-Voltage Test Pulse Sequence and making applicable adjustments, reset to 80 pulses for each probe pair, again review the outlined checklist in *Table 4-2*, and deliver therapeutic treatment.

4.6.8 Remove the Probes

Once the targeted area of the prostate has been treated, remove the probes and discard appropriately.

4.6.9 Post-Treatment

The Foley catheter will be left in place after the procedure and removed at the discretion of the treating physician. Any adverse events (AEs) will be recorded on the AE CRF.

It is anticipated that the majority of subjects will be discharged from the treatment facility the day of or the day after the procedure. Hospital discharge information will be entered on the CRF and any Adverse Events will be reported on the AE CRF.

It may be necessary for study subjects to be discharged from the hospital with the Foley catheter. If such is the case, a detailed set of instructions will be given to the subject on self-care.

Study subjects may be provided with a foam or blow-up donut to sit on which will take the pressure off the perineum. Study subjects will be prescribed levofloxacin 500 mg or another antibiotic of choice selected by the treating physician per institutional guidelines until catheter is removed and Minocycline 100 mg or another broad spectrum antibiotic of choice selected by the treating physician once the catheter is removed. Subjects will be instructed to use over-the-counter pain medications for the pain and discomfort they may experience. However, they should avoid using aspirin or ibuprofen for 24 hours, which may increase the chance of bleeding. If a stronger pain medication is needed, one can be prescribed.

Any medication (prescribed or over the counter) that the subject takes during his hospital stay will be recorded on the Concomitant Medication CRF.

4.6.10 Post-Procedure Follow-up

Following discharge from the hospital, subject follow-up will occur as outlined in *Table 4-3*, *Follow-up Visit Schedule*. At each visit, subjects undergo the procedures as described in the *Schedule of Subject Evaluations*, *Table 4-4*.

Table 4-3: Follow-Up Visit Schedule

Visit	Visit Window
Post-Procedure[1]	3-10 days post-treatment
1 Month	30 ± 7 days
3 Month	90 ± 14 days
6 Month	180 ± 14 days
9 Month	270 ± 14 days
12 Month	365 ± 28 days

1. This visit can be conducted over the phone, as long as urinalysis and MRI are not clinically indicated.

4.6.11 Quality of Life (QoL) Assessment Procedures

The following tools will be used to assess the subject's pre- and post-operative quality of life:

- Urinary Function Assessment: The subject's pre-and post-NanoKnife System treatment urinary function will be assessed at the baseline visit and at the 1, 3, 6, 9, and 12 month follow-up visits via the UCLA Expanded Prostate Cancer Index Composite[2] (UCLA-EPIC) Urinary domain and the International Prostate Symptom Score[3] (IPSS) and IPSS Quality of Life (IPSS-QoL) scores. Study subjects will be requested to complete the UCLA-EPIC and IPSS urinary function questionnaire to assess baseline urinary function within 30 days prior to the procedure. See **Appendix E – Subject Questionnaires – 1. Urinary Function Score** for more details on Questionnaire and Scoring instructions.
- Erectile Function Assessment: The subject's pre- and post-NanoKnife System treatment erectile function will be assessed at the Baseline visit and at the 1, 3, 6, 9, and 12 month follow-up visit via the International Index of Erectile Function-15[4] (IIEF-15) potency score. Study subjects will be requested to complete the IIEF-15 questionnaire to assess baseline erectile function within 30 days prior to the procedure. See **Appendix E – Subject Questionnaires – 2. Erectile Function Score** section for more details on Questionnaire and Scoring instructions.
- Quality of Life Assessment: The subject's pre- and post-NanoKnife System treatment overall quality of life will be assessed at the Baseline visit and at the 1, 3, 6, 9, and 12 month follow-up visit via 5 dimension scale EuroQol[5] (EQ-5D®). Study subjects will be requested to complete the EQ-5D® questionnaire to assess their baseline overall quality of life within 30 days prior to the procedure. See **Appendix E – Subject Questionnaires – 3. Quality of Life (QoL) Score** section for more details on Questionnaire and Scoring instructions

4.6.12 Physical Examination

All subjects will undergo a standard physical examination during the Baseline visit and at the 1, 3, 6, 9, and 12 month follow-up visits. The baseline physical exam should be completed within 30 days prior to treatment with the NanoKnife System.

On the day of treatment, the subject will be evaluated for any changes to physical exam as compared to the baseline assessment during an abbreviated physical exam prior to treatment with the NanoKnife System.

4.6.13 Laboratory Tests

Blood samples will also be collected at the Baseline visit and the 1, 3, 6, 9, and 12 month follow-up visits via standard forearm venipuncture, for assessment of serum PSA. Complete Blood Count will also be assessed at Baseline.

Urine samples will be collected pre-treatment, and at any subsequent follow-up visits if there is a question of an active urinary tract infection.

4.6.15 Radiologic Procedures

As the biopsy needles and NanoKnife electrode probes will be surgically placed into the prostate tissue under TRUS or MRI/TRUS fusion guidance, images of the prostate will be taken using this radiologic method to confirm the prostate is satisfactorily visible in sagittal and axial views.

Transrectal ultrasound will be conducted by the investigator according to standard accepted TRUS procedures. Subjects will be instructed to discontinue blood-thinning medications (e.g., aspirin, ibuprofen) for a week to 10 days prior to undergoing TRUS. Before undergoing the procedure, subjects will be instructed to drink a few glasses of water because a full bladder can improve visualization of the prostate gland. An enema may be administered to cleanse the bowel prior to the procedure.

Standardized measurements of the anterior/posterior, width and length of the prostate will be made. The measurements will be recorded on the ultrasound images maintained in the CRF. The ultrasound images will be saved in the study file.

The transrectal ultrasound procedure will also be used during the transperineal or transrectal prostate biopsy (at screening, at the 12-month follow-up visit, and if indicated by rising PSA) and during the NanoKnife System procedure.

mpMRI imaging of the prostate using utilizing the Prostate Imaging – Reporting and Data System Version 2.1 (PI-RADS version 2.1) will be conducted to document the location of the lesion and to characterize post-treatment effects and their correlation with outcome. The protocol including the recommended standardized protocol is presented in **Appendix A – Standard Operating Procedure: 1. mpMRI Protocol.**

4.6.16 Disease Assessment Procedures

The grade and location of the subject's prostate lesion will be assessed by the following procedures:

Determination of Prostate Specific Antigen (PSA):

Blood samples, as obtained from the above laboratory test procedure, will be used to determine the subject's serum PSA during the screening visit.

Serum PSA will also be assessed at the 1, 3, 6, 9, and 12 month follow-up visits to assess the effectiveness of therapy by measurement of prostate-specific antigen (PSA) kinetics including PSA nadir, time to PSA nadir and post-nadir PSA stability through 12 months post-treatment.

Study subjects who are found to be biochemical failures as defined by the Phoenix definition of nadir + 2 ng/mL, and subjects who have clinical suspicion, will be re-biopsied as explained below.

Transperineal or Transrectal Prostate Biopsy:

Pre-Treatment Biopsy and 12-Month Post-Operative Biopsy: The location and grade of the subject's prostate lesion will be documented via transrectal ultrasound-guided prostate biopsy (template mapping and/or targeted biopsy of lesion plus 10-14 core systematic sampling to include adequate sampling of the peripheral zone) for the screening biopsy and the biopsy performed at 12 months post-NanoKnife System procedure. The biopsy can be performed with either transperineal or transrectal biopsies to MR-visible lesions and 10-14 core systematic sampling to the peripheral zone. Three-dimensional data on the location and specific grade for each lesion is used for NanoKnife System ablation planning.[11]

Targeted transperineal or transrectal prostate biopsy will be carried out using general anesthetic or sedation/local anesthesia at the discretion of the clinician and also determined by subject choice. Antibiotic prophylaxis (Gentamicin and Cefuroxime or physician's preference) will be given at anesthetic induction – this is subject to change as per local microbiology advice and bacterial sensitivities. Subjects will only be discharged with a course of antibiotics (ciprofloxacin 500 mg twice daily for 5-7 days or physician's preference) if deemed at increased risk of sepsis (e.g., related to co-morbidities).

The 12-month biopsy will be in-field, in the area of the prostate treated with IRE. Biopsy of the untreated area will only be carried out if a new suspicious lesion is detected on the 12-month MRI, which was not present in that area in the pre-treatment MRI or if there is a suspicious change in another area.

Biopsy samples will be prepared according to institutional guidelines and sent to the pathology lab for analysis. The pathologist will be blinded to the type and location of the treatment of the subject's prostate. The pathologist will forward the results in the form of a pathology report to the primary investigator of the study.

For full details, see **Appendix A - Standard Operating Procedure: 2. Transperineal and Transrectal Prostate Biopsies Protocol**

Re-biopsy Prior to 12 Months: An early biopsy will also be performed in the event that there are positive findings on the 3-month mpMRI (PI-RADS 3 or higher lesion), a subject is deemed to have biochemical progression, or if the treating physician deems there to be clinical suspicion. Biochemical progression will be evaluated using the Phoenix definition of PSA nadir + 2 ng/mL. Subjects meeting this criterion, as well as subjects whose treating physician determines there is clinical concern, will be re-biopsied according to the baseline procedure (throughout entire volume of the prostate).

Positive Early In-Field Biopsy:

If the subject is re-biopsied, and there is a positive in-field core, this subject will be considered a failure for the primary efficacy endpoint (see Section 6.7). If the subject and the positive lesion still meet all of the inclusion criteria and none of the exclusion criteria[4] (i.e., the lesion is an intermediate-risk lesion of Gleason 3+4 or 4+3 and all other criteria are met), the subject can be re-treated with the NanoKnife System and will continue to be followed up for the duration of the study for secondary efficacy endpoints and safety. If the subject and their treating physician decide on re-treatment with something other than the NanoKnife System, or if they no longer meet the inclusion/exclusion criteria for the study (i.e., they have a Gleason ≥ 4+4 lesion and require whole-gland treatment), their post-re-treatment data will be excluded from the analysis.

Positive Early Out-of-Field Biopsy:

If the subject is re-biopsied, and there is a positive out-of-field core, this will not be considered a failure for the primary efficacy endpoint. If the subject and the new positive lesion still meet all of the inclusion criteria and none of the exclusion criteria[5] (i.e., the new lesion is an intermediate-risk lesion of Gleason 3+4 or 4+3 and all other criteria are met), the subject can be re-treated for the new lesion with the NanoKnife System as part of the study. If the subject and their treating physician decide on re-treatment with something other than the NanoKnife System, or if they no longer meet the inclusion/exclusion criteria for the study (i.e., the new lesion is Gleason ≥ 4+4 and the subject requires whole-gland treatment), they will exit the study though will continue to be followed for safety. These subjects who were re-treated with something other than the NanoKnife System for an out-of-field lesion prior to 12 months will have their post-re-treatment data excluded from the analysis.

Negative Early Biopsy:

If the subject is re-biopsied and there are no positive findings, they will continue in the study as planned, completing follow-up visits and the scheduled mpMRI and biopsy at 12 months.

If a subject is re-treated, regardless of the re-treatment selected, they should be followed up by the treating physician or their primary physician for routine urological care after the completion of the study.

Additional 'For Cause' Tests:

'For cause' additional tests such as ultrasound, mpMRI, CT scan, bone scan or PET/CT scan will be permissible. Specifically, an mpMRI at the 3-10 day visit is permitted if clinically indicated (i.e., if the patient is experiencing unexpected adverse events following treatment). 'For cause' biopsy is also permitted, if there is a clinical indication for this e.g., significant rise in PSA 9 – 12 months post-ablation.

Post-Treatment mpMRI:

3-month mpMRI: This will be used to verify that the treatment has been delivered appropriately according to the plan with all areas planned for coverage ablated, and to assess progression as well

[4] Note: Exclusion criterion #11 for prior or current prostate cancer therapies does not apply.
[5] Note: Exclusion criterion #11 for prior or current prostate cancer therapies does not apply.

as determining whether appropriate energy has been delivered. This will be performed at the 3-month visit to evaluate the extent and volume of necrosis of the prostate. PI-RADS version 2.1 guidelines and reporting will be utilized.

12-month mpMRI: This will follow the same protocol as the pre-treatment mpMRI during initial screening visit and the post-treatment mpMRI, and reported in the same manner.

See **Appendix A - Standard Operating Procedure: 1. mpMRI Protocol**.

Concomitant Medications and Therapies:

Concomitant medications and therapies will be assessed and recorded during each follow-up visit. These include:

- Urethral dilatation, cystoscopy, bladder neck incision (BNI), and transurethral resection of the prostate (TURP)
- Androgen suppression in any form, radiation therapy to the prostate in any form, focal/whole-gland cryotherapy, focal/whole-gland HIFU or radical prostatectomy
- Alpha blockade, 5 alpha-reductase inhibitors, anti-muscarinic medication
- Treatment-related antibiotics or pain medications

After the completion of 12-month follow-up, subjects will be referred to standard of care clinical follow up. AngioDynamics will be allowed to access EMR and similar medical records outside of the study through five years of follow up in order to assess long-term rate of progression, the need for re-intervention, and adverse events.

Table 4-4. Schedule of Subject Evaluations

Study Assessment	Visit 1	Visit 2	Visit 3		Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
	Screening	Baseline[3]	NanoKnife Treatment	Post-Procedure[10]	1 month 30 ± 7 days	3 months 90 ± 14 days	6 months 180 ± 14 days	9 months 270 ± 14 days	12 months 365 ± 28 days
Informed Consent[1]	X								
Enrollment	X								
Medical History	X								
Standard Physical Exam		X			X	X	X	X	X
Abbreviated Physical Exam			X						
Vitals Assessment[4]		X	X		X	X	X	X	X
CBC Panel		X							
Prothrombin Test (PT)		X							
Partial Thromboplastin Time (PTT)		X							
Urinalysis		X	X[9]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
Transrectal US	X		X						X
MRI protocols	X[2]			X[6]		X			X
Biopsy[7,8]	X[2]								X
PSA	X[2]				X	X	X	X	X
UCLA-EPIC Urinary Questionnaire		X			X	X	X	X	X
IPSS & IPSS-QoL Questionnaire		X			X	X	X	X	X
IIEF-15 Potency Questionnaire		X			X	X	X	X	X
EQ-5D Questionnaire		X			X	X	X	X	X
NanoKnife System Procedure			X						
Concomitant Medications		X	X	X	X	X	X	X	X
Adverse Event Assessment			X	X	X	X	X	X	X

1. Must be signed and dated prior to completing any study procedures.
2. Must be completed within 180 days prior to enrollment in the study. PSA for enrollment must be completed prior to biopsy, or must be taken more than 30 days after biopsy.
3. Complete within 30 days prior to NanoKnife System treatment.
4. Vitals assessments include height and weight, temperature, BP, respiration rate and pulse. Note, height only needs to be collected at Baseline.
5. Complete only if there is a question of an active UTI.
6. Complete only if clinically indicated (i.e., the subject is experiencing unexpected adverse events following treatment).
7. Transperineal or Transrectal Prostate Biopsy (template mapping and/or limited targeted).
8. Complete at additional time points if subject has a lesion ≥ PI-RADS 3 on the 3-month MRI, experiences biochemical failure as defined by the Phoenix criteria as nadir + 2 ng/mL, or upon clinical suspicion.
9. Complete pre-treatment, and at discharge only if there is a question of an active UTI.
10. Complete within 3 to 10 days of NanoKnife System treatment. This visit can be conducted over the phone, as long as urinalysis and MRI are not clinically indicated.

5. ADVERSE EVENTS DEFINITIONS AND REPORTING

5.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence, unintended disease or injury or any untoward clinical signs in subjects whether or not related to the Investigational Product and includes events related to the procedures involved. Safety events will refer to all adverse events (AEs), serious AEs (SAEs), adverse device effects (ADEs), and unanticipated adverse device effect (UADEs). This definition does not imply a relationship between the adverse event and the study procedure. The principal investigator will determine AE relationship (choices will be listed on the AE eCRF).

Any relevant medical conditions, problems, signs, symptoms and findings occurring prior to treatment are to be reported as pre-existing conditions and documented on the Medical History eCRF. If a pre-existing condition worsens during the follow-up (frequency increases and/or severity grade increases), it should be documented as an AE.

At each evaluation from the time of enrollment, the investigator or designee will determine whether an AE has occurred. The AE, date of onset, seriousness, severity, duration, treatment, outcome and relationship will be recorded on the AE eCRF. Adverse events will be assessed by a physician and monitored until they are resolved, stabilized, or the subject has reached study completion.

The term “severity” refers to the intensity of a specific event (CTCAE v5.0 will be used to determine this). The seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

All adverse events will be reported by the investigator and/or study team and reviewed by the sponsor in compliance with applicable regulations. Adverse events may be volunteered by subjects, elicited by the investigator or designee, or collected via observation by the investigator.

5.2 Serious Adverse Events

A serious adverse event (SAE) is defined as any event that:

- Leads to death
- Leads to serious deterioration in the health of a subject that:
    - Results in a life-threatening illness or injury
    - Results in a permanent impairment of a body structure or a body function
    - Requires in-subject hospitalization or prolongation of index hospitalization
    - Results in medical or surgical intervention to prevent permanent impairment to a body structure or a body function

All SAEs should be reported to the sponsor within 10 working days of learning of the event. The sponsor should be contacted via email or phone or by completing the AE eCRF (which triggers sponsor notification). SAEs should be followed until resolved, stabilized, or the subject has reached study completion.

The investigator is responsible for informing the IRB of any SAEs. Copies of SAE correspondence with the investigators, regulatory authorities, and sponsor must be retained with study records and provided to the sponsor, when applicable.

5.3 Adverse Device Effects

Adverse device effects (ADEs) are a subset of adverse events. The ADEs are only those AEs caused by or related to the NanoKnife System. This definition includes any event resulting from insufficiencies or inadequacies in instructions for use, the deployment, or any malfunction of the device, including any event that is a result of a use error or intentional misuse.

ADEs should be entered on the AE eCRF as described in Section 5.1 above. The principal investigator will determine relationship, severity, and all other information requested on the eCRF.

5.3.1 Device Complaint and Malfunction

For any event involving suspicion of a device malfunction, the sponsor may request that the clinical investigator return the device (when possible) for further evaluation. Device malfunctions or failures are not to be reported as an adverse event; however, if there is an adverse event that results from a device failure or malfunction, that specific event would be recorded on the eCRF as noted above.

5.4 Unanticipated Adverse Device Effect

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Investigators are required to submit a report of any suspected UADE as soon as possible, but not later than 10 working days after the investigator first learns of the effect. Additionally, reports must be provided to the reviewing IRB per national and local requirements.

When an investigator suspects an event meets the definition of a UADE, then the effect, date of onset, seriousness, severity, duration, treatment, outcome and relationship will be recorded on the AE eCRF.

The sponsor or sponsor's designee must then conduct an evaluation of the suspected UADE and report the results of the findings to the FDA and to all reviewing IRBs and participation investigators within 10 working days after the sponsor or sponsor's designee first received notice of the effect. Thereafter, the sponsor or sponsor's designee shall submit additional reports concerning the event as FDA requests. Reporting to the FDA will be consistent with 21 CFR Part 803.53 and 21 CFR Part 812.150.

5.5 Deaths

When a site becomes aware of a subject's death, it should be reported to the sponsor or sponsor's designee within 10 working days of becoming aware of the event, by way of completion of the

appropriate eCRF, phone, or email. The death should also be reported to the reviewing IRB per local and federal requirements.

5.6 Adverse Event Relatedness

The investigator will be responsible for making a determination on the causal relationship of the AE. Specifically, the investigator will report whether the AE was related to the study procedure or related to the study device.

The causal relationship for each adverse event will be rated as described in *Table 5-1*, below:

Table 5-1: Adverse Event Relatedness

Unrelated	The cause of the AE is known and is not related to any aspect of study participation including the underlying condition.
Possibly Related	There is a reasonable possibility that the event may have been caused by study participation. The AE has a timely relationship to the study procedure(s); however, it follows no known pattern of response and an alternative cause seems more likely or there is significant uncertainty.
Probably Related	It is probable that the event was caused by study participation. The AE has a timely relationship to the study procedure(s) and follows a known pattern of response, but a potential alternative cause may be present.
Definitely Related	The event was definitely related to study participation. A related event has a strong temporal relationship and an alternative cause is unlikely.

5.7 Adverse Event Severity

Adverse event severity will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 as described in *Table 5-2*, below:

Table 5-2: CTCAE Severity Grades

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.[6]
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated;

[6] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

	disabling; limiting self-care ADL.[7]
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

5.8 Adverse Event Coding

Adverse events will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Adverse events will be summarized by MedDRA system organ class and preferred terms.

5.9 Concomitant Medications Coding

Concomitant medications will be coded using the most recent version of the WHO Drug Dictionary Enhanced (WHO DDE).

5.10 Clinical Endpoint Committee (CEC)

The CEC is an independent body tasked with the review and adjudication of individual subject specific adverse events (AEs)/serious adverse events (SAEs) and/or clinical endpoint events that occur during the course of the investigative study. The CEC will consist of a minimum of three members with expertise in prostate health. None of the members will have direct involvement in the conduct of the study or have any conflicts of interest.

[7] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

6 STATISTICAL ANALYSIS

6.1 General Considerations

All data gathered in the study will be listed and presented by study site and subject number. All subjects enrolled will have data presented.

Data for continuous variables will presented using descriptive statistics including mean, median, standard deviation, minimum, and maximum. Categorical variables will be tabulated by visit and presented using descriptive statistics including frequencies and percentages. Confidence intervals will be displayed for success rates.

Safety evaluations will be based on the incidence, severity and type of AEs. AEs will be coded as specified in the protocol, graded by CTCAE v5.0 severity, and will be listed in by-subject data listings. Tabulation will also be provided that enumerates AEs by maximum severity. Deaths, SAEs, and events resulting in study discontinuation will be tabulated.

Change from baseline in clinical laboratory parameters will be summarized across time on study. Shift tables may be produced for selected laboratory parameters if implied by the data.

6.2 Determination of Sample Size

This study is designed to assess the efficacy of the NanoKnife System with respect to the primary efficacy endpoint (12-month negative in-field biopsy rate), as well as to assess the safety of the NanoKnife System with respect to the primary safety endpoint (rate of adverse events).

Assuming that 69% of the subjects undergoing treatment with the NanoKnife System will have an in-field negative 12-month biopsy, a minimum of 51 ITT subjects are required to achieve at least 80% power to conclude that the NanoKnife System's in-field negative biopsy rate is at least 52%.

The performance goal of 52% was based on the Sonablate 450 de novo summary (DEN150011), which reported a negative biopsy rate of 61% at 12 months (missing data imputed as positive), minus a 9% non-inferiority margin.

Table 6-1: Sample Size Determination

Endpoint	Primary Analysis Population	Assumed Performance of the NanoKnife System	Performance Goal	Non-Inferiority Margin	Power	Sample Size
12-month negative in-field biopsy rate	ITT	0.69	0.52	0.09	80%	51

For safety, it was determined that there is greater than an 80% chance to detect at least one medically significant adverse event if the true event rate is at least 1.6% with a sample size of 100 subjects.

Allowing for a possible 15% drop-out rate for subjects who will be lost to follow-up, a minimum of 118 subjects will be enrolled in the clinical study.

Note: the study is not powered to make claims regarding the secondary endpoints.

6.3 Analysis Populations

6.3.1 Intent-to-Treat (ITT) Population

The ITT population includes all subjects who were enrolled and treated with the NanoKnife System. Any subject who has a NanoKnife probe inserted will be considered treated for the ITT population. Note: Subjects who are enrolled but not treated will be documented as screen failures. The ITT population will serve as the primary analysis set for all safety and efficacy endpoints.

6.3.2 Per Protocol (PP) Population

The PP population includes all subjects who were enrolled and treated with the NanoKnife System, and who had no major protocol deviations. The PP population will serve as a supportive analysis set for all efficacy endpoints.

6.4 Missing Data

The following imputation methods will be used for the primary analysis of all endpoints:

- When calculating the proportion of subjects with a negative in-field biopsy, subjects with missing biopsy information post-ablation will be imputed as “positive”
- Subjects with missing pre- or post-ablation prostate volume measurements will be imputed as having a zero change in volume
- Missing PSA data will be imputed using last observation carried forward (LOCF)
- Missing data for IIEF-5, IPSS and EPIC questionnaires will be imputed using a multiple imputation approach at the item/question level.

All other endpoints/data (e.g., safety events) will be analyzed based on available (non-imputed) data, unless specified otherwise.

6.5 Subject Disposition

All subjects undergoing treatment as part of this study will be included in analyses, including those subjects for which treatment could not be completed due to adverse events or other complicating factors. Subjects will be followed for a maximum of 12 months, unless a subject has unresolved AEs, in which case they will be followed until all AEs are resolved or are determined to have no resolution, and disposition of all these subjects will be presented, including those completing follow-up and those

excluded due to treatment failure, adverse events, withdrawal by the subjects or Investigator, and those lost to follow-up.

6.6 Pooling

All tabulations will pool data across study sites. However, as stated above, data listing will be presented by individual study site.

6.7 Primary Effectiveness Endpoint

The primary efficacy endpoint is the rate of subjects with a negative in-field biopsy at 12 months. The number and proportion of subjects obtaining a negative in-field biopsy at 12 months will be presented, along with the 95% confidence interval of the proportion.

Subjects who are biopsied prior to 12 months (if there are positive findings on the 3-month mpMRI, if the subject is deemed to have biochemical progression, or if the treating physician deems there to be clinical suspicion) and have a positive in-field biopsy will be imputed as failures for the analysis of the primary efficacy endpoint. Note: Subjects with early positive out-of-field biopsies will not be considered failures for the primary efficacy endpoint.

6.8 Primary Safety Endpoint

The primary safety endpoint is the incidence of adverse events by type and CTCAE v5.0 severity through 12 months. The number and proportion of subjects who experienced at least one adverse event will be summarized, and the 95% confidence interval of the proportion will be presented.

If a subject is re-treated with something other than the NanoKnife System during the study, safety data collected during and after the re-treatment will not be included in the safety analyses.

6.9 Secondary Endpoints

Additional endpoints for statistical consideration will include the following:

- Rate of negative in-field biopsy at 12 months as defined by the Delphi consensus criterion of absence of clinically significant disease (≤ 3 mm of Gleason ≤ 6 disease in any biopsy core is insignificant)[1]
- Assessment of urinary function by comparison of pre- and post-operative UCLA Expanded Prostate Cancer Index Composite (UCLA-EPIC) Urinary Domain[2] and International Prostate Symptom Scores (IPSS)[3] and IPSS Quality of Life (IPSS-QoL) scores.
- Assessment of erectile function by comparison of pre- and post-operative 15-Item International Index of Erectile Function (IIEF-15)[4] potency scores.
- Effectiveness of therapy by measurement of prostate-specific antigen (PSA) kinetics including time to PSA nadir.
- Assessment of changes in prostate volume by comparison of pre-treatment and 12-month prostate volume measured via mpMRI.
- Assessment of ablation effectiveness by evaluation of prostate tissue by mpMRI at 3 months post-treatment and at 12 months post-treatment.

- Assessment of need for secondary or adjuvant treatment following treatment with the NanoKnife System.
- Evaluation of subject reported pre- and post-operative Quality of Life (QoL) using the 5-dimension scale EuroQol (EQ-5D®).[5]

If a subject is re-treated with something other than the NanoKnife System during the study, secondary endpoint data collected during and after the re-treatment will not be included in the secondary endpoint analyses.

Negative in-field biopsy at 12 months as defined by Delphi consensus criteria: Negative in-field biopsy is defined to be the absence of clinically significant disease (≤ 3 mm of Gleason ≤ 6 disease in any biopsy core is insignificant).[1] Prostate cancer that is ≤ 3 mm Gleason 6 will be considered clinically insignificant cancer and will not be considered a positive biopsy for this endpoint.

UCLA-EPIC[2]: The Urinary domain score from the UCLA-EPIC (University of California Los Angeles Expanded Prostate Cancer Index Composite) Urinary questionnaire will be presented for each visit (i.e., 1, 3, 6, 9, and 12 month follow-up). In addition, the 6-month and 12-month change from baseline will also be tabulated. See **Appendix E – Subject Questionnaires – 1.1 UCLA-EPIC** section for more details on the questionnaire and scoring instructions.

IPSS and IPSS-QoL[3]: The total score from the IPSS (International Prostate Symptom Score) and IPSS-QoL questionnaire will be presented for each visit (i.e., 1, 3, 6, 9, and 12 month follow-up). In addition, the 6-month and 12-month change from baseline will also be tabulated. See **Appendix E – Subject Questionnaires – 1.2 IPSS and IPSS-QoL** section for more details on the questionnaire and scoring instructions.

IIEF-15[4]: The IIEF-15 (International Index of Erectile Function 15 items) will have the Total Satisfaction Score and each of the 4 subscales (Erectile Function, Intercourse Satisfaction, Orgasmic Function, and Sexual Desire) will be presented for each visit (i.e., 1, 3, 6, 9, and 12 month follow-up). In addition, the 6-month and 12-month change from baseline will also be tabulated. See **Appendix E – Subject Questionnaires – 2.1 IIEF-15** section for more details on the questionnaire and scoring instructions.

PSA: The mean PSA levels at each visit, the overall percent reduction in PSA levels from baseline to each visit, and the proportion of subjects with a PSA reduction compared to baseline at each visit will be presented. Mean PSA nadir and post-nadir PSA values through 12 months post-treatment will be summarized.

Prostate Volume: The mean prostate volume at each visit, the overall percent reduction in prostate volume from baseline to each visit, and the proportion of subjects with reduction in prostate volume compared to baseline at each visit will be presented, as measured by mpMRI.

Evaluation of Prostate Tissue by mpMRI: The number and proportion of subjects determined to have cancer-bearing prostate tissue lesions on the 12-month mpMRI will be presented.

Secondary or Adjuvant Treatment: The number and proportion of subjects undergoing secondary or adjuvant treatment during the study will be presented.

EQ-5D[5]: The EQ-5D (EuroQoL – 5 Dimensions) will have the Total score and each of the 5 subscales (Mobility, Self-care, Usual activities, Pain/Discomfort, Anxiety/Depression) presented for each visit (i.e., 1, 3, 6, 9, and 12 month follow-up). In addition, the 6-month and 12-month change from baseline will also be tabulated. See **Appendix E – Subject Questionnaires – 3.1 EQ-5D** section for more details on the questionnaire and scoring instructions.

6.10 Exploratory Endpoint

Rate of progression, re-intervention, and adverse events: Once they complete 12-month follow-up, subjects will be referred to standard of care clinical follow up. AngioDynamics will be allowed to access EMR and similar medical records outside of the study through five years of follow up in order to assess long-term rate of progression, the need for re-intervention, and safety.

7 DATA HANDLING AND QUALITY ASSURANCE

7.1 Case Report Forms

This trial will utilize electronic Case Report Forms (eCRFs) to collect subject data via an electronic data capture (EDC) system. Case Report forms are presented in **Appendix B**.

The investigator is responsible for the accuracy and completeness of data reported on the eCRFs. Each set of eCRFs must be reviewed and signed by the investigator. The investigator also agrees to maintain accurate source documentation as part of the subject's medical records. These source documents may include chart notes, laboratory reports, images, etc. All eCRFs will be source data verified by the Clinical Research Associate (CRA) during regularly scheduled monitoring visits.

7.2 Subject Identifiers

All data used in the analysis and reporting of the study will be without identifiable reference to the subject. Only the unique subject number will be used to identify subject data submitted to the sponsor, and only the investigating site will be able to link the unique subject ID to the subject's name.

7.3 Study Monitoring Duties

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendments (s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Study monitors will conduct site initiation visits to train the study sites on the protocol prior to the study start. Interim monitoring visits will be conducted to check compliance with the protocol including completeness of informed consent forms and accuracy of entries on the eCRFs. Source documents will be verified where applicable. Study close-out visits will also be conducted per study monitoring plan, following all applicable regulatory requirements. Records of each visit will be documented in the appropriate monitoring report format and will include a statement of findings, conclusions, and any actions taken to correct any deficiencies noted during the visit. The monitor will report to the sponsor any non-compliance with the signed Investigator Statement, the study protocol, applicable GCP requirements, or any conditions imposed by the IRB. The frequency of monitoring visits will be determined by the sponsor.

The Sponsor or sponsor's designee, and the FDA may conduct site audits at any time during the study to ensure the validity and integrity of the data.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site (s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical study is conducted and data are generated and documented, and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The investigational site will provide direct access to all study related source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory requirements.

7.4 Investigator's Regulatory Binder

Investigators shall maintain a Regulatory Binder provided by the Sponsor.

7.5 Record Retention Period

Investigators shall maintain all study related documentation for a period of two years following completion of the study.

8 STUDY ETHICS AND CONDUCT

8.1 Role of the Sponsor

As the study sponsor of this clinical trial, AngioDynamics has the overall responsibility for the conduct of the study, including assurance that the study meets the requirements of the appropriate regulatory bodies. In this study, the sponsor will have certain direct responsibilities and may delegate other responsibilities to the CRO.

8.2 Ethical Conduct of the Study

The investigator agrees that the study will be conducted according to the applicable FDA regulations (21 CFR). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

8.3 Institutional Review Board (IRB)

Federal regulations, ISO 14155 and 21 CFR 56 require that approval be obtained from an IRB prior to participation of subjects in research studies. Prior to subject enrollment, a signed copy of the IRB approval letter must be submitted to AngioDynamics. In addition, the protocol, informed consent, advertisements to be used for subject recruitment, and any other written information regarding this study to be provided to the subject and/or the subject's legal authorized representative, must be approved by the IRB. Documentation of all IRB approvals will be maintained by the site and will be available for review by the sponsor or its designee.

All IRB approvals should be signed by the IRB chairperson or designee and must identify the IRB by name and address, the clinical protocol by title and/or protocol number, and the date approval was granted. The Investigator is responsible for submitting and obtaining initial and continuing review of the trial at intervals not exceeding 1 year or as otherwise directed by the IRB. The investigator must supply the sponsor or its designee written documentation of continued review of the study.

8.4 Confidentiality

A Subject Identification Log shall link the data collected during the study to source documents such as hospital records and medical charts. The Subject Identification Log shall be kept in a secure location with restricted access.

All information collected during the course of this study will be kept confidential, except that sponsor's representatives, local IRB and FDA will have access to this information.

The sponsor's representatives and/or FDA will have access to relevant medical charts or hospital files for purposes of source data verification. A HIPAA waiver shall be obtained as part of the Informed Consent Form from the IRB giving access to the source data for this purpose.

No information which could identify a subject will be used in reports or publications.

Subjects will remain anonymous for data analysis. Should the study require a future review of source documentation collected after official close-out (data collected after the 12-month follow-up visit), it will be necessary to obtain an additional informed consent and waiver of authorization for access to medical records from the IRB.

8.5 Subject Compliance

Subjects' participation in the study will terminate when they either complete the 12-month follow-up visit or discontinue participating in the study prior to completion. The reason for the subject's termination from the study will be documented on the Termination CRF. Upon study completion or termination, each subject will be instructed to visit his referring physician for evaluation and monitoring and/or treatment following the best practice guidelines for intermediate risk subjects.

Subjects may voluntarily withdraw from the study at any time, but a physician cannot arbitrarily remove a subject from the investigation. The reason for all subject withdrawals should be documented. If a subject is withdrawn from the study, every effort will be made to continue to follow the subject for safety, including the use of phone calls (up to three) and couriered mail, to obtain safety follow-up information.

Subjects will be required to meet the follow-up visit schedule outlined in *Table 4-4*.

8.6 Follow-Up

Subjects requiring follow-up will be reminded of their follow-up visits via telephone calls and a certified letter, if necessary. The investigator will keep a record of follow-up attempts in the Subject Follow-Up Log. A subject will be deemed lost-to-follow-up after the inability to obtain a response from the subject or legal representative on three separate occasions separated by at least one day. These contacts may be telephone calls or emails to the subject or legal guardian, or the primary care provider if the subject is unable to be contacted. However, at least one effort using a certified letter should be attempted at the last known address.

8.7 Medical Risk Analysis

Because the NanoKnife System is investigational within the prostate 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 associate with the use of the device and the associated clinical risks associated with the procedure (e.g., general anesthesia) along with the mitigations available to reduce this hazard. Potential risks associated with the use of the NanoKnife System include, but are not limited to, the risk outlined in *Table 8-1*.

Table 8-1: Potential Hazards

Hazard	Potential Effect(s)	Early Recognition	Mitigation
General anesthesia (procedural)	Aspiration-infection Pulmonary compromise-ARDS Urinary retention, suprapubic catheterization Paralysis Coma Death	Periodic assessment of airway patency, respiratory rate, and oxygen saturation. Routine monitoring of vital signs	Supervision by trained anesthesiologist Intra-operative monitoring Pre-op history and physical exam
Muscle Blockade (procedural)	Extended anesthesia (too much blockade) Insufficient anesthesia—pain Toxicity (If the organ that metabolizes the muscle block has compromised function)	Physical assessment and pharmacological management per ASA Standards	Pre-op history and physical exam Selection of appropriate agents. Patient management by Anesthesiologist.
Cardiac Arrhythmia (both procedural and device, unlikely from device due to distance)	Acute decrease in BP Fibrillation Cardioversion Death	The Anesthesiologist will be monitoring the patient continuously during procedure.	Anesthesia monitoring History and physical exam Availability of treatment drugs and cardioversion equipment; ECG monitoring and procedure pacing
Multiple Prostate Biopsies (procedural)	Bleeding Infection Hematoma Pain	Needle placement is performed using continuous image guidance.	Biopsy technique and physician training
IRE electrode needles placed in or through sensitive structures. (Foreseeable misuse)	Sharp trauma / perforation of structures. (Blood vessels, nerves, urethra, bowel, bladder). Bleeding Infection. Pain	Probe placement is performed using continuous image guidance. Patients will be monitored for hemodynamic instability	Physician training; product labeling
Insufficient Muscle Blockade (procedural)	Muscle strains or damage. Electrodes moved out of position. Disruption of drapes (contamination of sterile field) Trauma (to patient and medical staff) from flailing limbs.	Patient assessment by Anesthesiologist	Choice of agent and dose Subject observation Physician training

Hazard	Potential Effect(s)	Early Recognition	Mitigation
Vascular Dissection (device)	Nerve damage Hematoma Surgical intervention Transfusion Death	Probe placement is performed using continuous image guidance. Patients will be monitored for hemodynamic instability.	Proper positioning and imaging guidance Physician training
Perforation (device)	Infection Fever Sepsis Coagulopathy Death	Monitoring of patient condition for signs of sepsis, hemodynamic instability	Proper positioning and imaging guidance Physician training
Hemorrhage (device)	Hematoma Surgical intervention Transfusion Increased recovery time Death	Patients will be monitored for hemodynamic instability	Proper positioning and imaging guidance Physician training Observation of subject during procedure
Lack of Sterile Technique/Breach of Sterile Field (procedural)	Infection Abscess Fever Sepsis Coagulopathy Death	Monitoring of patient vital signs, monitoring for signs of sepsis.	Inspection of device packaging prior to use Sterilization and package integrity validation
ECG (EKG) Disruption after pulsing (2-3 sec)	Delayed intervention (can substitute arterial pulse until ECG returns)	The Anesthesiologist will be monitoring the patient continuously during procedure. The ECG monitoring device will alarm.	Validation of ECG pacing and interface if used
Nerve Damage (device)	Erectile Dysfunction	Probe placement is performed using continuous image guidance.	Nerve sparring nature of IRE; placement of needles and conduct of procedure using appropriate imaging observation
Acute or Sub-acute Vascular Damage (device)	Infarction /necrosis of non-targeted prostate tissues	Probe placement is performed using continuous image guidance. The NanoKnife generator provides precise control of probe voltage and duration, as well as automatic current limits and reporting of pulse currents.	Pre-procedure planning Physician training Use of imaging for guidance
Injury to Prostatic Urethra or External Sphincter (device)	Urinary incontinence Impotence Surgical repair Lifestyle changes	Probe placement is performed using continuous image guidance.	Pre-procedure planning Physician training Use of imaging for guidance

Hazard	Potential Effect(s)	Early Recognition	Mitigation
Urethro-rectal Fistula (procedural or device)	Pain Fever Abscess formation Infection, sepsis Surgical repair	Probe placement is performed using continuous image guidance. The NanoKnife generator provides precise control of probe voltage and duration, as well as automatic current limits and reporting of pulse currents.	Pre-procedure planning Physician training Use of imaging for guidance
Rectal Tear/ Perforation (from TRUS guided biopsy or Endorectal MRI coil) (procedural)	Pain Fever Abscess formation Infection, sepsis Surgical repair	Monitoring of patient condition for signs of sepsis.	Pre-procedure planning Physician training Use of imaging for guidance
Postoperative Hemorrhage (device)	Blood transfusion Pain Hematoma Medical/surgical intervention Death	Probe placement is performed using continuous image guidance. Patients will be monitored for hemodynamic instability.	Pre-procedure planning Physician training Use of imaging for guidance Avoidance of unnecessary needle insertion

Other accepted focal treatments for prostate neoplasia, 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 radical prostatectomy.

The NanoKnife System utilizes irreversible electroporation (IRE), a non-thermal ablation modality that uses short pulses of DC electric current to create irreversible pores in the cell membrane, thus causing cell death. This method 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. It is theorized that these advantages will also apply to use in the prostate.

8.8 Protocol Deviations

The investigator will not knowingly commit a major protocol deviation without prior written approval from the sponsor except in medical emergencies or in unforeseen, isolated instances where minor changes are made that will not increase the subject's risk or affect the validity of the trial. In medical emergencies, prior approval for protocol deviations will not be required, but the sponsor must be notified within two working days of the incident. Periodic monitoring of protocol compliance will be performed for each site. The sponsor has the right to suspend enrollment at sites deemed to have excessive protocol compliance issues.

All deviations related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment must be appropriately documented and reported. Other protocol deviations to

be considered include non-adherence to the protocol that results in a significant additional risk to the subject, or non-adherence to FDA regulations and/or ISO 14155.

The investigator must document and explain any protocol deviation in the subject's source documentation. The IRB should be notified of all protocol deviations in a timely manner. Protocol deviations should be reported to the IRB periodically, according to their requirements. Deviations will also be documented by the monitor during site visits and those observations will be reviewed with the investigator.

If the investigator believes that any exception to the protocol is justified for an individual subject or if the investigator has a question concerning a subject who may not meet an eligibility criterion, they should contact the sponsor's medical monitor. The sponsor will evaluate circumstances where the investigator deviates from the study protocol and will retain the right to remove either the investigator or the investigational site from the study.

The following deviations will be considered major deviations for the purposes of defining the Per Protocol analysis population:

- Subjects who are not consented for the trial
- Subjects for whom the intended ablation is not delivered
- Subjects who are not intermediate risk, defined as Gleason score 3+4 or 4+3, PSA ≤15 ng/mL or PSA density < 0.15 ng/mL^2 if PSA is > 15 ng/mL, and ≤ clinical stage T2c
- Subjects who miss three consecutive follow-up visits
- Subjects who have had prior or current prostate cancer therapies:
    - Biologic therapy for prostate cancer
    - Chemotherapy for prostate cancer
    - Hormonal therapy for prostate cancer within three months of procedure
    - Radiotherapy for prostate cancer
    - Surgery for prostate cancer

8.9 Study Reporting Requirements

The study will be registered on *www.ClinicalTrials.gov* as required by FDA.

By participating in this study, the investigator agrees to submit SAE reports according to the timeline and method outlined in this protocol. In addition, the investigator agrees to submit annual reports to his/her IRB as appropriate.

Upon completion or termination of the study, the principal investigator (PI) must submit a final written report to the sponsor and IRB. The report must be submitted within 3 months (90 days) of completion or termination of the trial. The sponsor will submit all reports required by the appropriate regulatory authorities, including unanticipated adverse device effects, withdrawal of IRB approval, list of current investigators, annual progress reports, recall information, final reports and protocol deviations.

8.10 Selection of Investigators

The sponsor will select qualified investigators, obtain a signed Investigator's Agreement and provide all investigators with the information necessary to conduct the study.

8.10.1 Financial Disclosure

Investigators and sub-investigators are required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under Title 21 CFR 54. In addition, the investigator must notify the sponsor promptly of any relevant changes that occur during the course of the study, at the completion of the study and 1 year following the completion of the study.

8.10.2 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ISO14155 and Title 21 CFR 812 by providing the following essential documents, including, but not limited to:

- An original investigator-signed Investigator Agreement page of the protocol
- An IRB-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject
- IRB approval of the investigator, protocol, and acknowledgement of the instruction manuals
- Curricula vitae (CV) for the PI and each investigator participating in the study. Current licensure must be noted on the CV or a copy of the license provided. CVs must be signed and dated by the investigators within 1 year of study startup, indicating that they are accurate and current.
- Financial disclosure information (as stated above) and a commitment to promptly update this information if any relevant changes occur
- Laboratory certifications and normal ranges for any local laboratories used by the site in accordance with Title 42 CFR 493

8.11 Site Training

The training of appropriate clinical site personnel will be the responsibility of the sponsor or its designee. To ensure proper device usage, uniform data collection, and protocol compliance, the sponsor or designee will present formal training sessions to relevant study site personnel. The sponsor reserves the right to enforce retraining for sites who have demonstrated study or procedure compliance issues.

8.12 Policy for Publication and Presentation of Data

The results of this study are intended to be published in a peer-reviewed journal and as abstracts/presentations at medical congresses under the oversight of a Multi-Disciplinary Publications Committee. All data and results and all intellectual property rights in the data and results derived from the study will be the property of AngioDynamics. Investigators must discuss any publication or

presentation with AngioDynamics prior to release and obtain written consent on the intended publication.

AngioDynamics recognizes the right of the investigator to publish the results upon study completion. However, the investigator must send a draft manuscript of the publication or abstract to AngioDynamics thirty (30) days in advance of submission in order to obtain approval prior to submission of the final version for publication. This will be reviewed promptly and approval will not be withheld unreasonably.

If after 180 days from the conclusion of the study, the Sponsor has not published the results, the investigators may publish without prior approval from the Sponsor. This includes the release of negative outcomes and if the study is terminated early results will be hastened.

In case of a difference of opinion between AngioDynamics and the investigator(s), the contents of the publication will be discussed in order to find a solution which satisfies both parties.

8.13 Study Governance

This protocol was developed in accordance with good clinical practices. It has been designed in collaboration with the Society of Urologic Oncology Clinical Trials Consortium (SUO-CTC) and other key stakeholders. SUO-CTC provided scientific and clinical oversight during protocol development and will continue to do so throughout the trial and in the development of the analysis plan. Study decisions that may affect the design or outcome of the trial will be reviewed with the SUO-CTC.

8.14 Impact on Medicare Beneficiaries

The National Cancer Institute estimates there will be 248,530 new cases and 34,130 deaths attributed to prostate cancer in the United States in 2021.[12] This disease impacts men of all ages, including the Medicare population; the median age at diagnosis is 67 years. It is estimated that 60% of new prostate cancer diagnoses occur in men over the age of 65 years.

This study enrolls men with prostate cancer over the age of 50, as long as they have greater than 10 years of life expectancy. The merits of the NanoKnife System will not impact only the Medicare beneficiary population, and will impact age groups younger and older than 65 years old.

8.15 Sponsor or Regulatory Agency Termination of Study

The sponsor reserves the right to cancel the study at any time for business or scientific reasons. Subjects who are actively enrolled in the study will continue to be followed until their participation is completed, but no new subjects will be enrolled.

APPENDIX A - STANDARD OPERATING PROCEDURE

1. mpMRI PROTOCOL

Please reference PI-RADS version 2.1.

2. TRANSPERINEAL AND TRANSRECTAL PROSTATE BIOPSIES PROTOCOL

The following format will be used to report all Transperineal template mapping biopsies:

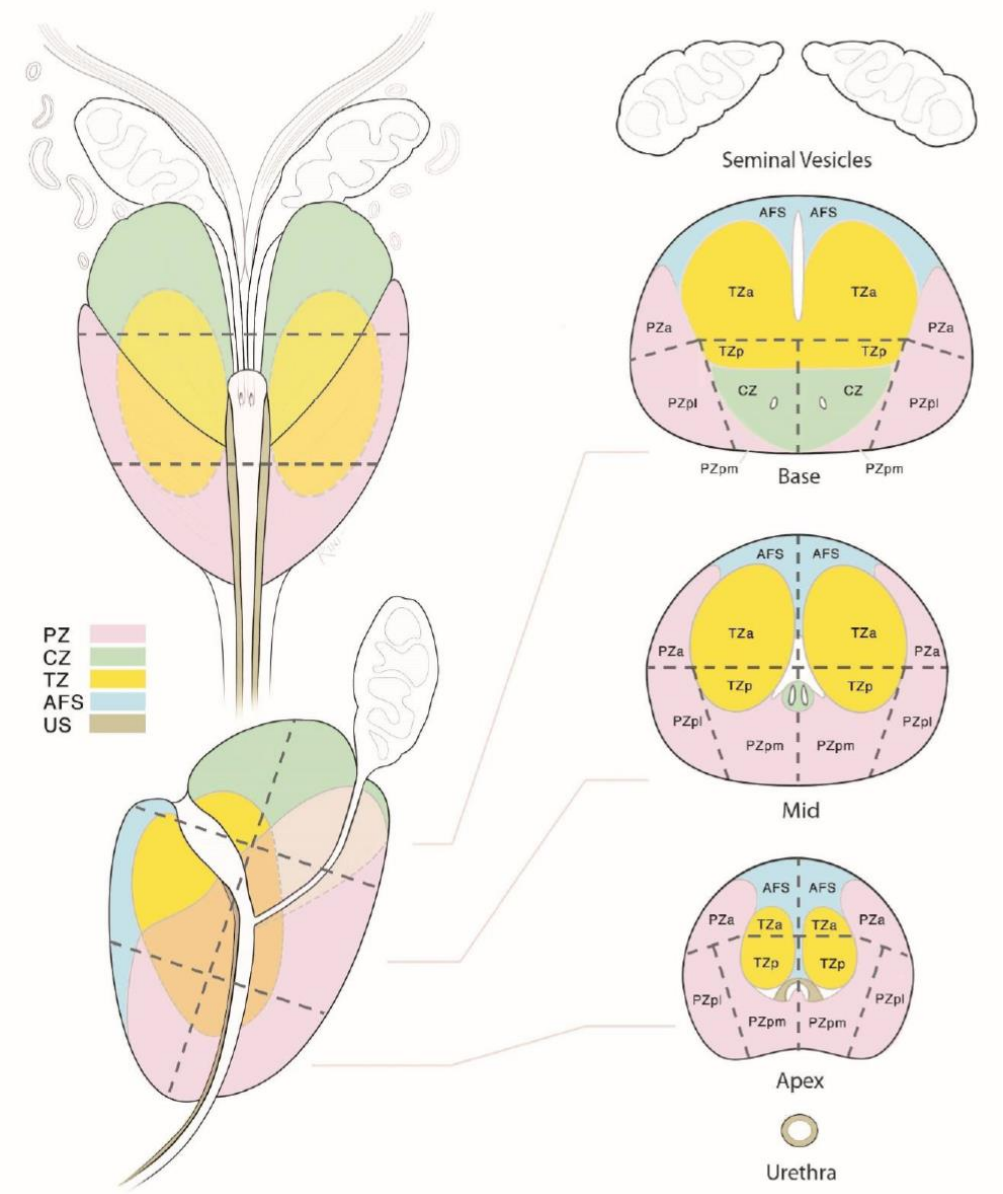

Figure 2.1 PI-RADS v2.1 Sector Map

Freehand or grid-assisted transperineal biopsy should include adequate (leave number up to PI discretion) targeted cores of any MRI visible lesion and systematic biopsy to include adequate sampling of the peripheral zone sectors (PZpm, PZpl, PZa) of PI-RADS 2.1 sector map. Systematic biopsies should include 10-14 cores with at least 2 cores from each peripheral zone sector with care taken to include both apical and basal sampling. In the event that the targeted lesion lies in one of

the PI-RADS v2.1 peripheral zone sectors, systematic sampling from that sector may be omitted, but the systematic biopsy should include at least 2 cores from each peripheral zone sector not sampled with targeted biopsies.

The following format will be used to report all Transrectal template mapping biopsies:

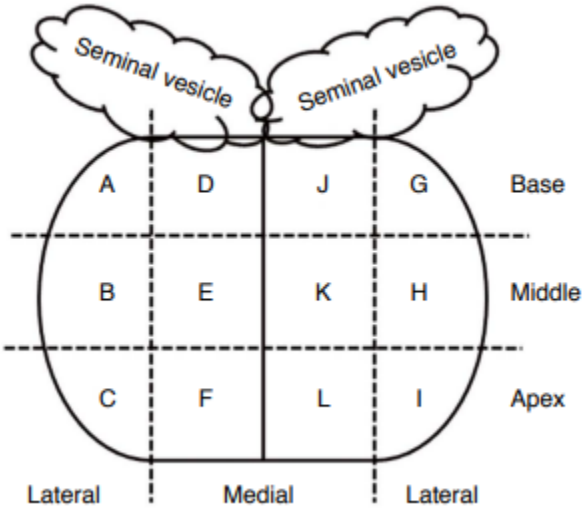

Figure 2.2 Transrectal 12-Core Biopsy Schematic

Schematic diagram of 12-core transrectal needle biopsy analysis. The prostate gland is divided into 12 compartments. A, right lateral base; B, right lateral middle; C, right lateral apex; D, right medial base; E, right medial middle; F, right medial apex; G, left lateral base; H, left lateral middle; I, left lateral apex; J, left medial base; K, left medial middle; L, left medial apex.

Transrectal biopsy should include adequate (leave number up to PI discretion) targeted cores of any MRI visible lesion and 12 core systematic biopsy to include all regions defined in Transrectal 12 core biopsy schematic (Figure 2.2). In the event that the targeted lesion lies in one Transrectal 12 core biopsy compartment, systematic sampling from that sector may be omitted.

3. *OPTIONAL MRI/TRUS FUSION IMAGE REGISTRATION FOR IRE TREATMENT PLANNING PROTOCOL*

Pre-Operative Treatment Planning

If MRI/TRUS fusion device is being used, manual contouring will be performed on the prostate capsule and the boundary of visible lesions (suspected to be cancer-bearing tissue) using special-purpose software. The lesion and prostate capsule will be delineated by the uro-radiologist and/or a trained urologist. Concordance for positive histology of contoured lesions will be verified on template mapping biopsies (TPM).

Peri-Operative Image Registration

Firstly, a standard set-up procedure will be performed with the NanoKnife System and TRUS devices, prior to IRE treatment. The area to be treated will then be planned by the operating surgeon i.e., according to pre-operative MRI and TPM findings and pre-operative discussion between the operating surgeon and the uro-radiologist, by stating the intended grid reference placement of the NanoKnife probes within the 5 mm brachytherapy grid. These grid references will be recorded at this stage.

Secondly, a 3D ultrasound volume file will be acquired and used to register the MRI and US images. The accuracy of the image alignment will be visually inspected by the surgeon and information of the registered location of the tumor saved to a file. The information acquired will be used to visually assess the extent of the planned treatment through comparison of grid references between TRUS and an aligned grid on the registration software. At this stage, the surgeon may adapt the treatment plan based on the information from the registration software. This may include a decrease or increase in treatment volume, through movement of the NanoKnife probes within the brachytherapy grid, with the constraint that the final image-registration-informed treatment volume may not exceed a predefined maximum volume of tissue (according to the 'dose-escalation' protocol). The operating surgeon will be free to reject the information provided by using the registration software if (s)he believes that this may compromise the subject treatment in any way. In this case, the surgeon will record the reasons, which will be stored with the other data collected as part of the study. The updated grid references will be recorded at this stage, if a change has been made to the treatment plan.

Post-Operative Image Registration

Using the same method described above, registration will be used to register post-operative MR images with the TRUS volume acquired at the end of the procedure. The spatial distribution of the treated tissue (i.e., necrosis), visible in the MR images, will then be compared with the treatment plan and the treated tissue determined by analysing the final TRUS image.

APPENDIX B - CASE REPORT FORM

APPENDIX C - INFORMED CONSENT FORM

APPENDIX D - NANOKNIFE™ SYSTEM USER MANUAL

APPENDIX E – SUBJECT QUESTIONNAIRES

1. Urinary Function Score

The following tools will be used to assess the subject's preoperative and postoperative urinary function and symptoms at baseline as well as at the 1, 3, 6, 9, and 12 month follow-up visit.

1.1 UCLA-EPIC: University of California Los Angeles Expanded Prostate Cancer Index Composite

UCLA-EPIC is a subject-validated prostate cancer HRQoL instrument that complements prior instruments such as UCLA-PCI by measuring a broad spectrum of urinary, bowel, sexual, and hormonal symptoms, thereby providing a robust tool for comprehensive assessment of HRQoL issues important in contemporary prostate cancer management.[2]

In this study, the UCLA-EPIC Urinary Domain Only Questionnaire will be administered, not the full UCLA-EPIC questionnaire.

1.1.1 UCLA-EPIC Questionnaire Urinary Domain Only Questionnaire

The clinical trial sites will use the latest version of UCLA-EPIC Urinary Domain Only Questionnaire, which can be accessed on the questionnaire website here (UCLA-EPIC website). Below is the most recent version of the UCLA-EPIC Urinary Domain Only Questionnaire.[13]

EPIC

The Expanded Prostate Cancer Index Composite

Urinary Assessment

This questionnaire is designed to measure Quality of Life issues in patients with Prostate cancer. To help us get the most accurate measurement, it is important that you answer all questions honestly and completely.

Remember, as with all medical records, information contained within this survey will remain strictly confidential.

Today's Date (please enter date when survey completed): Month_____Day_____Year_____

Name (optional): _____

Date of Birth (optional): Month_____Day_____Year_____

Do Not Mark in This Space

URINARY FUNCTION

This section is about your urinary habits. Please consider **ONLY THE LAST 4 WEEKS**.

1. Over the **past 4 weeks**, how often have you leaked urine?

   More than once a day..... 1
   About once a day..... 2
   More than once a week..... 3 (Circle one number) 23/
   About once a week..... 4
   Rarely or never..... 5

2. Over the **past 4 weeks**, how often have you urinated blood?

   More than once a day..... 1
   About once a day..... 2
   More than once a week..... 3 (Circle one number) 24/
   About once a week..... 4
   Rarely or never..... 5

3. Over the **past 4 weeks**, how often have you had pain or burning with urination?

   More than once a day..... 1
   About once a day..... 2
   More than once a week..... 3 (Circle one number) 25/
   About once a week..... 4
   Rarely or never..... 5

4. Which of the following best describes your urinary control **during the last 4 weeks**?

   No urinary control whatsoever..... 1
   Frequent dribbling..... 2 (Circle one number) 26/
   Occasional dribbling..... 3
   Total control..... 4

Do Not Mark in This Space

5. How many pads or adult diapers per day did you usually use to control leakage **during the last 4 weeks?**

None	0	
1 pad per day	1	
2 pads per day	2	(Circle one number)
3 or more pads per day	3	27/

6. How big a problem, if any, has each of the following been for you **during the last 4 weeks?**
(Circle one number on each line)

		No Problem	Very Small Problem	Small Problem	Moderate Problem	Big Problem	
a.	Dripping or leaking urine	0	1	2	3	4	28/
b.	Pain or burning on urination	0	1	2	3	4	29/
c.	Bleeding with urination	0	1	2	3	4	30/
d.	Weak urine stream or incomplete emptying	0	1	2	3	4	31/
e.	Waking up to urinate	0	1	2	3	4	32/
f.	Need to urinate frequently during the day	0	1	2	3	4	33/

7. Overall, how big a problem has your urinary function been for you **during the last 4 weeks?**

No problem	1		
Very small problem	2		
Small problem	3	(Circle one number)	34/
Moderate problem	4		
Big problem	5		

THANK YOU VERY MUCH!!

1.1.2 UCLA-EPIC Scoring Instructions

The clinical trial sites will use the latest version of UCLA-EPIC Scoring Instructions, which can be accessed on the questionnaire website here (UCLA-EPIC website). Below is the most recent version of the UCLA-EPIC Scoring Instruction.[14]

Please refer to the questionnaire website for the most updated scoring guideline instructions.

Scoring Instructions for the Expanded Prostate cancer Index Composite (EPIC)*

To request a copy of **EPIC** or related information, please contact any of the following investigators:

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Mark S. Litwin, M.D., M.P.H.
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Los Angeles, CA 90095-1738
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Email: mlitwin@mednet.ucla.edu

* A SAS macro for computing the EPIC scores is available at the EPIC web site:
http://roadrunner.cancer.med.umich.edu/epic/

Questions regarding the SAS macro should be addressed to:

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The **Expanded Prostate cancer Index Composite (EPIC)** was developed by researchers at University of Michigan and UCLA to measure health related quality of life among men with prostate cancer.[1] It represents an adaptation of the UCLA Prostate Cancer Index,[2] modified to enhance sensitivity to therapy effects by increasing the number of prostate-targeted items to 50 (compared to 20 in the original UCLA-PCI). EPIC has been validated in men with localized prostate cancer who underwent surgery, external beam radiation, or brachytherapy with or without the use of hormonal adjuvants. EPIC is sensitive to specific HRQOL effects of these therapies and to HRQOL effects of cancer progression.[3]

EPIC assesses the disease-specific aspects of prostate cancer and its therapies and comprises four summary domains (Urinary, Bowel, Sexual and Hormonal). Factor analysis supports dividing the Urinary Domain Summary Score into two distinct *Incontinence* and *Irritative/Obstructive* subscales. In addition, each Domain Summary Score has measurable *Function Subscale* and *Bother Subscale* components. Response options for each EPIC item form a Likert scale, and multi-item scale scores are transformed linearly to a 0-100 scale (see following page: EPIC scoring), with higher scores representing better HRQOL. A summary of EPIC Summary Score and Subscale characteristics are tabulated (from Ref. 1):

HRQOL Domain	Number of items	Mean Score (sd)	Test-retest reliability	Internal consistency reliability
HRQOL Domain Summary Scores				
Urinary	12	80.2 (17.5)	0.88	0.88
Bowel	14	86.6 (15.7)	0.84	0.92
Sexual	13	33.1 (23.6)	0.91	0.93
Hormonal	11	86.6 (13.8)	0.80	0.82
Domain-Specific HRQOL Subscales				
Urinary Subscales				
Function	5	86.5 (16.7)	0.83	0.69
Bother	7	75.8 (20.4)	0.87	0.85
Incontinence*	4	83.2 (22.9)	0.87	0.89
Iritative/Obstructive*	7	79.7 (18.5)	0.85	0.81
Bowel Subscales				
Function	7	87.9 (13.6)	0.78	0.75
Bother	7	85.3 (18.8)	0.85	0.90
Sexual Subscales				
Function	9	29.5 (24.0)	0.90	0.92
Bother	4	41.1 (30.1)	0.78	0.84
Hormonal Subscales				
Function	5	84.0 (15.3)	0.79	0.51
Bother	6	88.7 (13.6)	0.73	0.73

* A single global urinary bother item, which does not distinguish bother related to incontinence from that related to urinary obstruction, is not included in the Urinary Incontinence or Urinary Irritative/Obstructive subscales; therefore, 11 urinary items comprise these 2 subscales whereas the Urinary Summary Domain includes 12 items.

EPIC can be used alone or combined with other instruments, including the AUA-SI, FACT-P, and Medical Outcomes Study SF-12 or SF-36. Inter-scale correlation between EPIC and these instruments has indicated that efficient (yet comprehensive) HRQOL assessment can be achieved by co-administering EPIC with SF-12.[4] Concurrent use of the AUA-SI can also provide useful complementary clinical information.[5] The following scoring instructions therefore assume that EPIC will be co-administered with SF-12 and the AUA-SI, with these 3 instruments combined according to the following format:

SF-12 General Health Function Survey (first 12 items):	Items 11-22
EPIC (subsequent 50 items excluding 7 AUA-SI items):	Items 23-34, 42-79
AUA Symptom Index (7 items embedded in EPIC Urinary Section):	Items 35-41

Scoring the EPIC

There are 2 steps involved in scoring EPIC:

Step 1. The response for each item is standardized to a 0 to 100 scale according to the table below.

Item Number	Item Response Value	Standardized Value
23,24,25,42,43,48,56,57,58,60,61, 62,63,64,69,70,71,72	1	0
	2	25
	3	50
	4	75
	5	100
26,59	1	0
	2	33
	3	67
	4	100
27	0	100
	1	67
	2	33
	3	0
28,29,30,31,32,33,49,50,51,52,53, 54,65,66,67,74,75,76,77,78,79	0	100
	1	75
	2	50
	3	25
	4	0
34,44,45,46,55,68	1	100
	2	75
	3	50
	4	25
	5	0
47	1	100
	2	50
	3	0
73	1	0
	2	50
	3	100
	4	50
	5	0

Step 2. Using the item groupings listed below for each HRQOL Domain Summary Score or Subscale score, average the standardized values (see Step 1, above) for all items within a group to create the summary or subscale score. (If ≥20% of the items that comprise a domain summary score or subscale score are missing a response, the corresponding domain summary or subscale score can not be calculated).

To calculate the following HRQOL domain Summary Score or Subscale Score:	Determine the average of the Standardized Values (see Step 1, above) for the following items:	Number of non-missing items needed to compute score (otherwise, set score to missing)
HRQOL Domain Summary Scores		
Urinary Summary	23-34	10
Bowel Summary	42-55	12
Sexual Summary	56-68	11
Hormonal Summary	69-79	9
Domain-specific HRQOL Subscales		
Urinary Subscales:		
Function	23-27	4
Bother	28-34	6
Incontinence	23,26-28	4
Irritative/Obstructive	24,25,29-33	6
Bowel Subscales:		
Function	42-48	6
Bother	49-55	6
Sexual Subscales:		
Function	56-64	8
Bother	65-68	4
Hormonal Subscales:		
Function	69-73	4
Bother	74-79	5

Please note:

- Item numbers are indicated along the right border of the questionnaire (question numbers on left of questionnaire pages are not used for scoring because some questions contain multiple items).
- The **AUA Symptom Index** (AUA-SI) is included to provide a clinical context for EPIC urinary measures.[5] However, the seven AUA-SI items are not included in the calculation of any EPIC domain scores. For ease of reference, scoring instructions for the AUA-SI are provided in the Appendix below.
- The **Medical Outcomes Study SF-12** is a validated measure of General Health Function developed by RAND.[4] It is intended to be used here to derive 2 summary scores (physical and mental component summaries) relevant to general HRQOL status, which provide a context for EPIC score results. The SF-12 items themselves are not included in the calculation of any EPIC domain scores. For ease of reference, scoring instructions for the SF-12 are provided in the Appendix below.
- Optional satisfaction and socio-demographic/medical history items (Items 80-117) from the original UCLA-PCI can be administered with EPIC. These items are not included in any EPIC domain score calculations.

REFERENCES

(1) Wei JT, Dunn R, Litwin M, Sandler H, Sanda MG. Development and Validation of the Expanded Prostate Cancer Index Composite (EPIC) for Comprehensive Assessment of Health-Related Quality of Life in Men with Prostate Cancer. Urology 2000; In press.

(2) Litwin MS, Hays RD, Fink A, Ganz PA, Leake B, Brook RH. The UCLA Prostate Cancer Index: development, reliability, and validity of a health-related quality of life measure. Medical Care 1998; 36(7):1002-1012.

(3) Sanda MG, Dunn RL, Sandler HM, McLaughlin PW, Montie JE, and Wei JT. Comparison of HRQOL after brachytherapy, radical prostatectomy, or external beam radiation for localized prostate cancer. ASCO Proceedings 2000; 19:327a.

(4) Ware JE, Keller SD, Kosinski M. SF- 12: How to Score the SF-12 Physical and Mental Health Summary Scales. Second ed. Boston: The Health Institute, New England Medical Center, 1995.

(5) Barry MJ, Fowler FJ, Jr., O'Leary MP, Bruskewitz RC, Holtgrewe HL, Mebust WK et al. The American Urological Association symptom index for benign prostatic hyperplasia. The Measurement Committee of the American Urological Association. Journal of Urology 1992; 148(5):1549-57; discussion 1564.

APPENDIX: Scoring the AUA-SI and SF-12

Scoring the AUA Symptom Index:

The sum of the raw values for items 35-41 provides the total AUA symptom score.[5]

Scoring the Medical Outcomes Study SF-12:

There are 3 steps involved in calculating the SF-12 Physical Component Summary (PCS) and Mental Component Summary (MCS) scores.

Step 1. Check for missing or out-of-range values for items 11-22 (SF-12 portion of combined survey). If any missing or out-of-range values are found for those items, the PCS and MCS scores can not be calculated.

Step 2. Convert each item response into both physical and mental standardized values according to the table on the following page.

Step 3. Sum the physical standardized values from step 2 across all 12 items and add 56.57706 to create the SF-12 PCS score. Sum the mental standardized values in similar fashion and add 60.75781 to create the SF-12 MCS score.

Converting SF-12 Item Responses to Physical and Mental Standardized Values:

Item Number	Item Response Value	Physical Standardized Value	Mental Standardized Value
11 (General Health)	1	0	0
	2	-1.31872	-0.06064
	3	-3.02396	0.03482
	4	-5.56461	-0.16891
	5	-8.37399	-1.71175
12 (Moderate Activities)	1	-7.23216	3.93115
	2	-3.45555	1.86840
	3	0	0
13 (Climbing Several Flights of Stairs)	1	-6.24397	2.68282
	2	-2.73557	1.43103
	3	0	0
14 (Accomplish less than you would like)	1	-4.61617	1.44060
	2	0	0
15 (Limited in the kind of activities)	1	-5.51747	1.66968
	2	0	0
16 (Accomplish less than you would like)	1	3.04365	-6.82672
	2	0	0
17 (Didn't do activities as carefully as usual)	1	2.32091	-5.69921
	2	0	0
18 (Pain interferes with normal work)	1	0	0
	2	-3.80130	0.90384
	3	-6.50522	1.49384
	4	-8.38063	1.76691
	5	-11.25544	1.48619
19 (Felt calm and peaceful)	1	0	0
	2	0.66514	-1.94949
	3	1.36689	-4.09842
	4	2.37241	-6.31121
	5	2.90426	-7.92717
	6	3.46638	-10.19085
20 (Have a lot of energy)	1	0	0
	2	-0.42251	-0.92057
	3	-1.14387	-1.65178
	4	-1.61850	-3.29805
	5	-2.02168	-4.88962
	6	-2.44706	-6.02409
21 (Felt downhearted and blue)	1	4.61446	-16.15395
	2	3.41593	-10.77911
	3	2.34247	-8.09914
	4	1.28044	-4.59055
	5	0.41188	-1.95934
	6	0	0
22 (Health interferes w/social activities)	1	-0.33682	-6.29724
	2	-0.94342	-8.26066
	3	-0.18043	-5.63286
	4	0.11038	-3.13896
	5	0	0

1.2 IPSS: International Prostate Symptom Score

The IPSS is an eight-question written screening tool used to screen for, rapidly diagnose, track the symptoms of, and suggest management of the symptoms of benign prostatic hyperplasia (BPH).[3] The 7-point symptom score of the IPSS was originally developed by the American Urological Association as AUA7 – with the subsequent addition of the single quality of life question.[15] The score consists of two parts:

- The first features seven questions regarding the severity of a range of urinary symptoms – three storage symptoms (urgency, frequency and nocturia) and four voiding symptoms (weak stream, hesitancy, intermittency and straining) – scoring from 1 to 5 for each question to make a maximum total score of 35.
- This is followed by a single item which addresses the impact of these symptoms on the subject's quality of life.[15]

1.2.1 IPSS and IPSS-QoL Scoring Instructions

Each question allows the subject to choose from one of six answers indicating severity of the particular symptom. Each answer is assigned a point from zero (0) to five (5). The total score can range from 0 to 24 (asymptomatic to very symptomatic).[15]

Allows stratification of the total symptom score into:

- mild (total IPSS 0-7)
- moderate (8–19)
- severe (20–35)

The IPSS-QoL question allows the subject to choose from one of seven answers indicating how they feel about maintaining their current urinary condition for the rest of their life. Subjects select a score from zero (0) indicating Delighted to six (6) indicating Terrible.

Please refer to the questionnaire website for the most updated and complete scoring guideline instructions.

1.2.2 IPSS and IPSS-QoL Questionnaire

The clinical trial sites will use the latest version of IPSS and IPSS-QoL Questionnaire, which can be accessed on the questionnaire website here (IPSS website). Below is the most recent version of the UCLA-EPIC Urinary Domain Only Questionnaire.[16]

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INTERNATIONAL-PROSTATE SYMPTOM SCORE (I-PSS)

INTERNATIONAL-PROSTATE SYMPTOM SCORE (I-PSS)							
	Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	
1. Over the past month, how often have you had a sensation of not emptying your bladder completely after you finished urinating?	0	1	2	3	4	5	
2. Over the past month, how often have you had to urinate again less than two hours after you finished urinating?	0	1	2	3	4	5	
3. Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
4. Over the past month, how often have you found it difficult to postpone urination?	0	1	2	3	4	5	
5. Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5	
6. Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5	
	None	1 time	2 times	3 times	4 times	5 or more times	
7. Over the past month, how many times did you most typically get up to urinate from the time you went to bed at night until the time you got up in the morning?	0	1	2	3	4	5	
Total I-PSS Score S =							
QUALITY OF LIFE DUE TO URINARY SYMPTOMS							
	Delighted	Pleased	Mostly satisfied	Mixed about equally satisfied and dissatisfied	Mostly dissatisfied	Unhappy	Terrible
1. If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that?	0	1	2	3	4	5	6
Quality of life assessment index L =							

2. Erectile Function Score

The following tools will be used to assess the subject's pre-operative and post-operative erectile function and symptoms at baseline as well as at the 1, 3, 6, 9, and 12 months follow-up visit.

2.1 IIEF-15: International Index of Erectile Function 15 items

The International Index of Erectile Function (IIEF) is a widely used, multi-dimensional self-report instrument for the evaluation of male sexual function. It has been recommended as a primary endpoint for clinical trials of erectile dysfunction (ED) and for diagnostic evaluation of ED severity. The IIEF was developed in conjunction with the clinical trial program for sildenafil, and has since been adopted as the 'gold standard' measure for efficacy assessment in clinical trials of ED.[17]

2.1.1 IIEF-15 Questionnaire

Below is the 1997 original version of the IIEF-15 Questionnaire.[18]

APPENDIX

Individual items of International Index of Erectile Function Questionnaire and response options (US version)

Question*	Response Options
Q1: How often were you able to get an erection during sexual activity? **Q2**: When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	0 = No sexual activity 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q3: When you attempted sexual intercourse, how often were you able to penetrate (enter) your partner? **Q4**: During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	0 = Did not attempt intercourse 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q5: During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	0 = Did not attempt intercourse 1 = Extremely difficult 2 = Very difficult 3 = Difficult 4 = Slightly difficult 5 = Not difficult
Q6: How many times have you attempted sexual intercourse?	0 = No attempts 1 = One to two attempts 2 = Three to four attempts 3 = Five to six attempts 4 = Seven to ten attempts 5 = Eleven+ attempts
Q7: When you attempted sexual intercourse, how often was it satisfactory for you?	0 = Did not attempt intercourse 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always

Q8: How much have you enjoyed sexual intercourse?	0 = No intercourse 1 = No enjoyment 2 = Not very enjoyable 3 = Fairly enjoyable 4 = Highly enjoyable 5 = Very highly enjoyable
Q9: When you had sexual stimulation or intercourse, how often did you ejaculate? **Q10**: When you had sexual stimulation or intercourse, how often did you have the feeling of orgasm or climax?	0 = No sexual stimulation/intercourse 1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q11: How often have you felt sexual desire?	1 = Almost never/never 2 = A few times (much less than half the time) 3 = Sometimes (about half the time) 4 = Most times (much more than half the time) 5 = Almost always/always
Q12: How would you rate your level of sexual desire?	1 = Very low/none at all 2 = Low 3 = Moderate 4 = High 5 = Very high
Q13: How satisfied have you been with your overall sex life? **Q14**: How satisfied have you been with your sexual relationship with your partner?	1 = Very dissatisfied 2 = Moderately dissatisfied 3 = About equally satisfied and dissatisfied 4 = Moderately satisfied 5 = Very satisfied
Q15: How do you rate your confidence that you could get and keep an erection?	1 = Very low 2 = Low 3 = Moderate 4 = High 5 = Very high

** All questions are preceded by the phrase "Over the past 4 weeks."*

2.1.2 IIEF-15 Scoring Instructions

The clinical trial sites will use the latest version of IIEF-15 Scoring Instructions, which can be accessed on the questionnaire website here (IIEF website).[18]

An example IIEF-15 scoring guide is presented below. Please refer to the questionnaire website for the most updated and complete scoring guideline instructions.

Scoring of the Instrument:
Responses are recorded on a Likert-type scale with response choices over a 5 or 6 point scale for items 1-10 and 11-15 respectively. The lower anchor for the 6 point Likert-type scale is 0 while the highest rating is 5. The 5 point Likert-type scale ranges from 1 to 5 with 1 representing the lower anchor.

The items are not weighted and total scores range from 5 to 75. The domain scores are computed by summing the scores for each individual item in each domain.
The sub-scales scores range as follows:

Erectile Function: 1-30
Orgasmic Function: 0-10
Sexual Desire: 2-10
Intercourse Satisfaction: 0-15
Overall Satisfaction: 2-10

On the Erectile Function sub-scale lower scores indicate *worse* erectile dysfunction, while on the remaining sub-scales higher scores indicate *less* dysfunction.

Interpretation and Analysis of Missing Data
All items have to be completed. A missing response to an item makes both the item score and the sub-scale score missing.

3. Quality of Life (QoL) Score

The following tool will be used to assess the subject's pre-operative and post-operative quality of life at baseline as well as at the 1, 3, 6, 9, and 12 months follow-up visit.

3.1 EQ-5D-5L: EuroQoL – 5 Dimensions

EQ-5D is a standardized measure of health status developed by the EuroQol Group in order to provide a simple descriptive profile and a single generic index value for health status that can be used in clinical and economic evaluations.[5,19]

The EQ-5D-5L level version (EQ-5D-5L) was introduced in 1990. The EQ-5D-5L essentially consists of 2 pages - the EQ-5D-5L descriptive system and the EQ visual analogue scale (EQ VAS). The EQ-5D-5L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The EQ VAS records the respondent's self-rated health on a vertical, visual analogue scale where the endpoints are labelled 'Best imaginable health state' and 'Worst imaginable health state.' This information can be used as a quantitative measure of health outcome as judged by the individual respondents. [5,19]

3.1.1 EQ-5D-5L Questionnaire

The clinical trial sites will use the latest version of EQ-5D-5L, which can be accessed on the questionnaire website here (EuroQol Website). Below is the questionnaire from the user guide of EQ-5D-5L published in September 2019.[19]

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Figure 1 / UK (English) EQ-5D-5L Paper Self-Complete (sample version)

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

- I have no problems in walking about ❑
- I have slight problems in walking about ❑
- I have moderate problems in walking about ❑
- I have severe problems in walking about ❑
- I am unable to walk about ❑

SELF-CARE

- I have no problems washing or dressing myself ❑
- I have slight problems washing or dressing myself ❑
- I have moderate problems washing or dressing myself ❑
- I have severe problems washing or dressing myself ❑
- I am unable to wash or dress myself ❑

USUAL ACTIVITIES (*e.g. work, study, housework, family or leisure activities*)

- I have no problems doing my usual activities ❑
- I have slight problems doing my usual activities ❑
- I have moderate problems doing my usual activities ❑
- I have severe problems doing my usual activities ❑
- I am unable to do my usual activities ❑

PAIN / DISCOMFORT

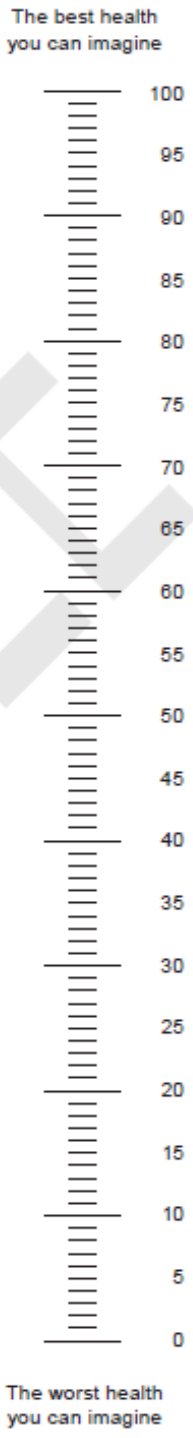
- I have no pain or discomfort ❑
- I have slight pain or discomfort ❑
- I have moderate pain or discomfort ❑
- I have severe pain or discomfort ❑
- I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

- I am not anxious or depressed ❑
- I am slightly anxious or depressed ❑
- I am moderately anxious or depressed ❑
- I am severely anxious or depressed ❑
- I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



3.1.2 EQ-5D-5L Scoring Instructions

Below are the scoring instructions from version 5.1 of EQ-5D-5L published in September 2019.[20] Please refer to the questionnaire website for the most updated and complete scoring guideline instructions.

2.1 / What is a health state?

Each of the five dimensions comprising the EQ-5D descriptive system is divided into five levels of perceived problems:

LEVEL 1: indicating no problem
LEVEL 2: indicating slight problems
LEVEL 3: indicating moderate problems
LEVEL 4: indicating severe problems
LEVEL 5: indicating unable to/extreme problems

A unique health state is defined by combining one level from each of the five dimensions.

A total of 3125 possible health states is defined in this way. Each state is referred to by a 5-digit code. For example, working clockwise from the top of the diagram, state 12345 indicates no problems with mobility, slight problems with washing or dressing, moderate problems with doing usual activities, severe pain or discomfort and extreme anxiety or depression, while state 11111 indicates no problems on any of the five dimensions.

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2. Scoring the EQ-5D-5L descriptive system

This example shows how a health state is described using the EQ-5D-5L descriptive system:

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

		Levels of perceived problems are coded as follows:
I have no problems in walking about	☑	☑
I have slight problems in walking about	☐	☐ Level 1 is coded as a '1'
I have moderate problems in walking about	☐	☐
I have severe problems in walking about	☐	☐
I am unable to walk about	☐	☐

SELF-CARE

I have no problems washing or dressing myself	☐	☐
I have slight problems washing or dressing myself	☑	☑ Level 2 is coded as a '2'
I have moderate problems washing or dressing myself	☐	☐
I have severe problems washing or dressing myself	☐	☐
I am unable to wash or dress myself	☐	☐

USUAL ACTIVITIES (*e.g. work, study, housework, family or leisure activities*)

I have no problems doing my usual activities	☐	☐
I have slight problems doing my usual activities	☐	☐ Level 3 is coded as a '3'
I have moderate problems doing my usual activities	☑	☑
I have severe problems doing my usual activities	☐	☐
I am unable to do my usual activities	☐	☐

PAIN/DISCOMFORT

I have no pain or discomfort	☐	☐
I have slight pain or discomfort	☐	☐ Level 4 is coded as a '4'
I have moderate pain or discomfort	☐	☐
I have severe pain or discomfort	☑	☑
I have extreme pain or discomfort	☐	☐

ANXIETY/DEPRESSION

I am not anxious or depressed	☐	☐
I am slightly anxious or depressed	☐	☐ Level 5 is coded as a '5'
I am moderately anxious or depressed	☐	☐
I am severely anxious or depressed	☐	☐
I am extremely anxious or depressed	☑	☑

This example identifies the health state '12345'.

Notes:

- There should be only ONE response for each dimension
- Missing values are preferably coded as '9'.
- Ambiguous values (e.g. two boxes are ticked for a single dimension) should be treated as missing values.
- This example is for the EQ-5D-5L Paper Self-Complete. Instructions for the interview and proxy versions are provided with those instruments.

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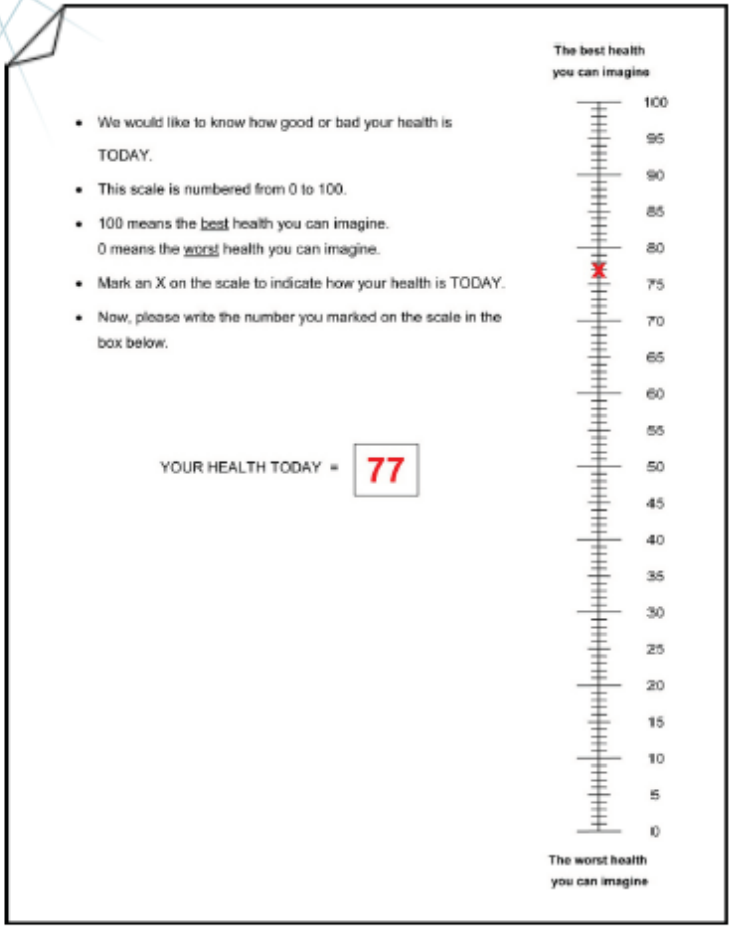
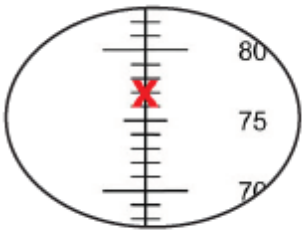
3. Scoring the EQ VAS

This example from the EQ-5D-5L Paper Self-Complete version shows how the EQ VAS is scored.





For example, the response above should be coded as 77

Notes:

- For this example, the response should be coded as 77
- Missing values should be coded as '999'.
- If there is a discrepancy between where the respondent has placed the X and the number he/she has written in the box, administrators should use the number in the box (this is only relevant for the Paper Self-Complete version).

PROTOCOL REVISION HISTORY

Version	Location	Summary of Change	Rationale for Change
2.0	Protocol Summary, Study Objectives; Protocol Summary, Secondary Endpoints; 3.2 Design; 3.3.2 Secondary Objectives; 3.5.2 Secondary Endpoints; 6.9 Secondary Endpoints	Removed rates of biochemical and clinical progression as a secondary objective and secondary endpoint, as clinically meaningful progression is already assessed by our primary endpoint.	Biochemical and clinical progression will still be monitored by the treating physician in order to determine if early follow-up biopsy is needed prior to the 12 month visit, but this will not be assessed as an objective or endpoint.
2.0	Protocol Summary, Inclusion Criteria; 4.3.1 Inclusion Criteria	Inclusion criterion "Has no evidence of extraprostatic extension or seminal vesicle invasion by mpMRI" split into two separate criteria: "Has no evidence of extraprostatic extension by mpMRI" and "Has no evidence of seminal vesicle invasion by mpMRI, and if suspected, confirmed by biopsy." Added requirement to confirm no evidence of seminal vesicle invasion by biopsy if suspected by mpMRI.	Clarifies criteria for evaluating evidence of extraprostatic extension and seminal vesicle invasion, to confirm that these are not present in study patients.
2.0	Protocol Summary, Inclusion Criteria; 4.3.1 Inclusion Criteria	Added "and in the judgement of the physician, the study is in the best interest of the subject" to the criterion "Has signed a written informed consent form."	Allows treating physician to determine whether participation in the study is in the best interest of the patient.
2.0	Protocol Summary, Exclusion Criteria; 4.3.2 Exclusion Criteria	Changed exclusion criterion "Had a malignancy within 5 years, including malignant melanoma, except for prostate cancer or other types of skin cancer" to "Had active treatment for a malignancy within 3 years, including malignant melanoma, except for prostate cancer or other types of skin cancer."	No safety impact expected from this change; allows a larger patient population by including clinically insignificant cancers.
2.0	Protocol Summary, Secondary Endpoints; 3.5.2 Secondary	Added "Rate of negative in-field biopsy at 12 months as defined by the Delphi consensus criterion of absence of clinically significant disease (≤ 3 mm of Gleason ≤ 6	Change adds an additional secondary endpoint in order to assess tissue ablation. No effect on patient safety or data collection;

	Endpoints; 6.9 Secondary Endpoints	disease in any biopsy core is insignificant)" as a secondary endpoint.	12 month biopsy is already a study activity for the primary endpoint.
2.0	Protocol Summary, Study Procedure	Updated requirement for the initial enrollment biopsy to be within 180 days of the NanoKnife treatment to be within 180 days of enrollment into the study.	Allows more flexibility in scheduling a procedure date post-enrollment, however biopsy and PSA for enrollment must still be less than or equal to 180 days old.
2.0	2 Study Device Overview, Table 2-1	Removed incorrect and duplicate entries from the Pulse Interval, Un-sycn row.	Administrative change.
2.0	3.2.1 Identifying the Disease by Multiparametric MRI	Added criteria for evaluating extraprostatic extension and seminal vesical invasion for inclusion into the study.	Clarifies criteria for evaluating evidence of extraprostatic extension and seminal vesicle invasion, to confirm that these are not present in study patients.
2.0	3.2.2 Disease Localization: Transperineal or Transrectal Prostate Biopsies	Clarified that the PSA value for inclusion into the study is to have been performed prior to the initial enrollment biopsy.	Administrative change.
2.0	4.5 Procedure Preparation	Removed sentence stating subjects will be scheduled for treatment with NanoKnife within 180 days of screening biopsy as this no longer applies (see change described in Sequence 7).	Allows more flexibility in scheduling a procedure date post-enrollment, however biopsy and PSA for enrollment must still be less than or equal to 180 days old.
2.0	4.6 Study Procedure	Clarified that institutional guidelines should be followed regarding pre-operative heparin to align with clinical practice.	There are multiple risk factors for DVT in this patient population (older, lithotomy position, pelvic treatment, cancer); change clarifies that institutional guidelines should be followed where no guidance was provided before.
2.0	4.6.9 Post-Treatment	Made the provision of a foam or blow-up donut to take pressure off the perineum optional.	Allows physicians to determine whether this is necessary due to a patient's post-procedure discomfort.
2.0	4.6.9 Post-Treatment	Removed sentence recommending avoidance of aspirin or ibuprofen for 24 hours post-treatment to align with clinical practice.	Very limited bleeding issues with transperineal treatments; allows physicians to prescribe pain medication per clinical practice.
2.0	4.6.12 Physical Examination	Removed "full" from "full standard physical exam" to clarify that psychiatric evaluation is not required.	Administrative clarification to remove potential that a psychiatric evaluation would unnecessarily be performed.

2.0	4.6.13 Laboratory Tests	Removed Complete Blood Count assessments for post-baseline follow-up visits.	Complete Blood Count not required post-baseline as it provides no clinical utility.
2.0	4.6.16 Disease Assessment Procedures	Updated definition of biochemical failure from the ASTRO definition of three consecutive rises in PSA to the Phoenix definition of nadir + 2 ng/mL to align with focal therapy best practices.	Using the Phoenix definition is more appropriate for focal therapy practice; ASTRO criteria is used for whole gland therapy.
2.0	4.6.16 Disease Assessment Procedures	Updated definition of biochemical failure from the ASTRO definition to the Phoenix definition, and clarified that patients whose physicians determine have clinical suspicion of recurrence will be re-biopsied.	Using the Phoenix definition is more appropriate for focal therapy practice; ASTRO criteria is used for whole gland therapy. Added clarity that patients who are suspected to have recurrence as determined by the physician can be re-biopsied prior to 12 months.
2.0	4.6.16 Disease Assessment Procedures, Table 4-4	Updated Table 4-4 Schedule of Subject Evaluations to reflect changes made throughout the protocol and described above.	See impact discussion for each respective change above.
2.0	6.3.1 Intent-to-Treat (ITT) Population	Defined treated with the NanoKnife System for the purposes of defining the ITT population, to be any subject who has a NanoKnife probe inserted. Added that patients who are enrolled but not treated will be considered screen failures and replaced.	Clarifies ITT population by defining how “treated with the NanoKnife System” is determined, and that subjects who are enrolled but do not move on to treatment will be a screen failure.
2.0	8.8 Protocol Deviations	Added subjects who are enrolled in the trial but do not receive the full intended treatment as a major protocol deviation.	Clarifies the Per Protocol population, to exclude patients who do not receive the intended ablation.
2.0	8.13 Policy for Publication and Presentation of Data	Added text regarding the intent to publish findings of the study in a peer-reviewed journal, to allow investigators to publish results without prior approval if the Sponsor has not done so within 180 days of the conclusion of the study, and to release negative outcomes and if the study is terminated early results will be hastened.	Clarifies the publication intent and release of information if there are negative outcomes or if the study is terminated.
2.0	8.15 Impact on Medicare Beneficiaries	Updated to change “men of all ages” to “over the age of 50” to reflect the inclusion criterion of the study.	Administrative change.

2.0	Appendix E. Subject Questionnaires, 3. Quality of Life (QoL) Score	Updated from EQ-5D-3L to the EQ-5D-5L.	EQ-5D-5L is a newer and more sensitive version of the EQ-5D-3L and is recommended for use in clinical trials by NICE.
3.0	Protocol Summary, Inclusion Criteria; 3.2 Design; 3.2.2 Disease Localization; 4.3.1 Inclusion Criteria; 4.6.16 Disease Assessment Procedures; Appendix A.2. Transperineal and Transrectal Prostate Biopsies Protocol	Updated the biopsy requirements for the study to include a range of 10-14 core systematic biopsy: a transperineal or transrectal targeted prostate biopsy of the lesion, plus a 10-**14** core systematic biopsy.	Added a range to the biopsy requirements to allow for additional sampling of the prostate, and to accommodate different hospital biopsy templates, whether transperineal or transrectal.
3.0	Protocol Summary, Inclusion Criteria; 4.3.1 Inclusion Criteria	Inclusion criterion #10 was updated to clarify: "A visible lesion on mpMRI that is accessible to Irreversible Electroporation (IRE) treatment (Note: **If prostate cancer is** detected via systematic standard biopsy **outside of the MRI visible lesion it** will not be considered an exclusion criterion provided the **positive core** is singularly located in the contralateral hemisphere of the prostate; is Gleason 6; and comprises no more than 6 mm linear extent of prostate-bearing tissue in a single core on standard biopsy)."	Clarifies wording for evaluating evidence of a secondary Gleason 6 insignificant cancer lesion in the contralateral hemisphere.
3.0	Protocol Summary, Exclusion Criteria; 4.3.2 Exclusion Criteria	Exclusion criterion #10 was updated to state: "Is unable **or unwilling** to catheterize."	Clarified that subjects who are unable or unwilling to have a catheter placed, for any reason, will be excluded from the study as catheterization is required for participation.
3.0	Protocol Summary, Secondary Endpoints; Protocol Summary, Study Schedule; 3.2.1 Identifying the Disease by Multiparametric MRI; 3.5.2 Secondary Endpoints; 4.6.1 Measure the Prostate; 4.6.16 Disease Assessment	Moved the mpMRI assessment from 3-10 days to 3-months post-treatment, but allow treating physicians to perform the 3-10 day mpMRI if clinically indicated (i.e., if subjects are experiencing unexpected adverse events).	Moving the mpMRI from 3-10 days to 3 months allows physicians to have an earlier assessment of disease progression, recurrence, and response to treatment, rather than rely only on PSA for a full 12 months. The timing of this assessment at 3 months corresponds with a recent publication on standardized surveillance methodologies after focal therapy written by an international multidisciplinary

	Procedures; Table 4-4 Schedule of Subject Evaluations; 6.9 Secondary Endpoints		consensus, which states that there should be a post-treatment imaging assessment within 6 months of treatment.[8] With mpMRI at 3 months, treating physicians will be able to view the ablation zone and obtain feedback on their technique, whereas the 3-10 day mpMRI was too soon after treatment for physicians assess treatment success.
3.0	3.2.1 Identifying the Disease by Multiparametric MRI	Added Likert scale for scoring the presence of seminal vesicle invasion on mpMRI, and clarified criterion for including the subject in the study (Inclusion Criterion #7).	Adding quantitative criteria for determining the presence of seminal vesicle invasion, to ensure that no subjects are enrolled who have this contraindicated characteristic of the disease.
3.0	4.6.10 Post-Procedure Follow-Up; Table 4-4 Schedule of Subject Evaluations	Changed the window for the 3-month visit from ± 7 days to ± 14 days to allow for time to schedule the 3-month mpMRI.	The window for the 3-month visit was widened to allow for scheduling of the 3-month mpMRI now at the 3-month visit. There is no impact to subject safety or data collection as this month-long window corresponds to guidance regarding the timing of post-focal therapy follow-up.
3.0	4.6.13 Laboratory Tests; Table 4-4 Schedule of Subject Evaluations	Removed the requirement for subjects to undergo urinalysis at discharge; urinalysis is now required at discharge if a UTI is suspected to correspond with the remaining post-treatment follow-up visits.	Subjects will still undergo urinalysis at discharge if there is suspicion of UTI, however, patients will be catheterized at discharge so requiring a urinalysis if not medically necessary would be difficult and onerous to the site and the subject.
3.0	4.6.16 Disease Assessment Procedures; Table 4-4 Schedule of Subject Evaluations	Added additional detail describing the re-treatment paradigm for subjects who are biopsied prior to 12 months and have positive findings.	Subjects who are found to have a positive biopsy prior to the 12-month assessment can be re-treated while remaining on the study for continued follow up and safety assessments, regardless of whether the subject is re-treated with IRE or another type of therapy.
3.0	4.6.16 Disease Assessment Procedures	Clarified that one of the purposes of the 3-month mpMRI is to assess progression.	The 3-month mpMRI will allow for the treating physician to assess disease progression prior to the 12-month mpMRI and biopsy.

[8] Lebastchi AH, George AK, Polascik TJ, et al. Standardized Nomenclature and Surveillance Methodologies After Focal Therapy and Partial Gland Ablation for Localized Prostate Cancer: An International Multidisciplinary Consensus. European Journal of Urology. 2020;78:371-378. https://doi.org/10.1016/j.eururo.2020.05.018

3.0	6.2 Determination of Sample Size	Clarified that the study is not powered to make claims regarding the secondary endpoints.	Administrative change.
3.0	6.7 Primary Effectiveness Endpoint; 6.8 Primary Safety Endpoint; 6.9 Secondary Endpoints	Updated descriptions of the primary efficacy endpoint, primary safety endpoint, and secondary endpoints to address the scenario in which patients are biopsied prior to 12-months and have positive findings.	Clarified impact of positive biopsy findings prior to the 12-month biopsy assessment.
3.0	Appendix A.2 Transperineal and Transrectal Prostate Biopsies Protocol	Updated Appendix A.2 Transperineal and Transrectal Prostate Biopsies Protocol to add instructions specific to transperineal template mapping biopsies (this was previously omitted in error).	Administrative change to provide guidance for transrectal biopsies which was previously omitted in error.
3.1	8.10 Selection of Investigators; 8.12 Device Returns	Removed references to device shipments and returns.	Administrative change.
3.1	Protocol Summary; 3.2 Design	Corrected references to pilot study to pivotal study.	Administrative change.
4.0	Protocol Summary, Inclusion Criteria; 4.3.1 Inclusion Criteria	Inclusion criterion #8 updated to clarify, "Physician is able to visualize prostate gland adequately on transrectal ultrasound imaging during **qualifying biopsy**."	Clarifies that the TRUS at screening is part of qualifying biopsy and a separate TRUS is not expected to be performed for enrollment.
4.0	Protocol Summary, Inclusion Criteria; 4.3.1 Inclusion Criteria	Inclusion criterion #9 was updated for grammar.	Administrative change.
4.0	Protocol Summary, Inclusion Criteria; 4.3.1 Inclusion Criteria	Inclusion criterion #10 was updated to clarify: "A visible lesion on mpMRI that is accessible to Irreversible Electroporation (IRE) treatment **(Note: prostate cancer detected via systematic standard biopsy outside of the adjacent sextant location of the MRI visible lesion will meet entry criterion provided the positive core is Gleason 6; has fewer than 3 prostate biopsy fragments/cores positive and ≤50% cancer in each of those fragments/cores on standard biopsy**."	Clarifies wording for evaluating evidence of secondary Gleason 6 insignificant cancer outside the target lesion. Prior wording was unclear as to the number of positive cores that were allowable; change limits to 2 cores.
4.0	Protocol Summary, Inclusion Criteria;	Inclusion criterion #11 was updated for grammar.	Administrative change.

	4.3.1 Inclusion Criteria		
4.0	Protocol Summary, Exclusion Criteria; 4.3.2 Exclusion Criteria	Exclusion criterion #11 was updated to clarify, "Has had **any** prior or current prostate cancer therapy, **including**: a) Biologic therapy for prostate cancer b) Chemotherapy for prostate cancer c) Hormonal therapy for prostate cancer within three months of procedure d) Radiotherapy for prostate cancer e) Surgery for prostate cancer."	Clarifies that all prior or current prostate cancer therapies are exclusionary.
4.0	Protocol Summary, Exclusion Criteria; 4.3.2 Exclusion Criteria	Exclusion criterion #15 was updated to clarify, "Is actively bleeding, has a bleeding disorder, **or is unable to interrupt blood thinning medications as clinically indicated per pre-operative best practices**."	Clarifies that patients who are on blood thinning medications but can interrupt them prior to treatment will not be excluded.
4.0	Protocol Summary, Exclusion Criteria; 4.3.2 Exclusion Criteria	Exclusion criterion #16 was updated to clarify, "Is a member of a vulnerable population, such as **cognitively impaired or incarcerated, that could expose them to undue influence, coercion, or inability to obtain informed consent**."	Updated to add more inclusive language.
4.0	Protocol Summary, Study Procedure; 4.2 Informed Consent; 4.3 Eligibility Criteria	Updated to define enrollment to occur at time of informed consent.	Administrative change.
4.0	3.2.2 Disease Localization: Transperineal or Transrectal Prostate Biopsies; Table 4-4 Schedule of Subject Evaluations	Updated to allow enrollment PSA to be taken after enrollment biopsy as long as it is taken greater than 30 days after biopsy.	Updated so as not to require patients to be re-biopsied if enrollment PSA is older than 180 days and needs to be reassessed.
4.0	4.4 Baseline	Updated to clarify that Screening and Baseline visits may be combined.	Administrative change.
4.0	4.4 Baseline; 4.6.11 Quality of Life (QoL) Assessment Procedures; 4.6.12	Updated to allow 30 days between Baseline visit and IRE treatment.	Updated to account for scheduling of the NanoKnife procedure and to account for postponements in treatment.

	Physical Examination; Table 4-4 Schedule of Subject Evaluations		
4.0	4.5 Procedure Preparation	Updated assessments to be performed prior to procedure—previously urinalysis and blood draw had been included in error. Bowel preparation updated to remove 1-2 hour requirement to conform with standard practice.	Administrative change.
4.0	4.6.10 Post-Procedure Follow-up; Table 4-4 Schedule of Subject Evaluations	Added footnote to allow Post-Procedure (3-10 days post-treatment) to be conducted over the phone to assess for Adverse Events and Concomitant Medications as long as urinalysis and MRI are not clinically indicated.	Updated to take into account COVID protocols at sites preferring virtual visits when possible as well as patient willingness to come back into the hospital.

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Statistical Analysis Plan (SAP)

Protocol Title:	Pivotal Study of the NanoKnife System for Ablation of Prostate Tissue in an Intermediate-Risk Patient Population
Protocol Version No./Date:	4.0/20-Jan-2023
CRF Version No./Date:	10.0/14-Feb-2023
SAP Version No./Date:	2.0/06-Jun-2023

1.0 Approvals

Sponsor	
Sponsor Name:	AngioDynamics
Representative/Title:	Juan Carlos Serna/Senior Vice President, Scientific and Clinical Affairs
Signature/Date:	Juan Carlos Serna Juan Carlos Serna 20 Jun 2023 15:37:40 UTC (Z) REASON: I approve this document ef63affd-a082-4739-be7f-50acd0b6164b
Representative/Title:	Ariel Bloch/Data Scientist
Signature/Date:	Ariel Bloch Ariel Bloch 06 Jun 2023 13:25:18 UTC (Z) REASON: I approve this document 12a1a6a5-e65d-47ef-8eb5-a8b673cebc59
Representative/Title:	Liz Manning/Director, Clinical Affairs
Signature/Date:	Liz Manning Liz Manning 07 Jun 2023 11:58:56 UTC (Z) REASON: I approve this document 318b00cf-1244-4b9b-bfdc-72fd6516d6d9
ICON	
Biostatistician/Title:	Pablo Hernández Alonso/Senior Biostatistician
Signature/Date:	Pablo hernandezalonso Pablo Hernandez Alonso 06 Jun 2023 11:18:59 UTC (Z) REASON: I approve this document de7d7923-a9b7-4988-80ab-81d8e0231150

2.0 Change History

Version/Date	Change Log
0.1/13-Dec-2021	Initial draft to AngioDynamics
0.2/08-Feb-2022	Updated after AngioDynamics review: • Section 9 renamed to "Endpoints" instead of "Endpoints and Covariates". • "Missing Data" section clarified. • "Demographic and Baseline Characteristics" section clarified. • Coding dictionaries versions updated. • All protocol deviations will be presented, instead of just important protocol deviations. • Transrectal ultrasound will not be presented. • Additional NanoKnife procedure information will now be presented in tables, not only listings. • Gleason scores for patients that do have a positive biopsy added for both negative in-field biopsy tables. • Paired t-test added for urinary and erectile function change from baseline assessments. • Screening *versus* Baseline clarifications, • Nadir definition added. • Adverse events *versus* adverse device events clarification. • Adverse event relationship to NanoKnife system procedure categories updated. • Physical examination analysis description updated.
1.0/23-Mar-2022	Updated after AngioDynamics review of version 0.2: • CRF version 8.0 dated 10Feb2022 included. • Agreed on the statement regarding multiple imputation within Section 6.1 Changes from Protocol. • Agreed on to remain Prostate volume within section 8.0 Study Design. • "Re-treatment with IRE or first re-treatment with other type" information was included in SAP and TFLs where needed. • 95% CI decimal places was included in section 12.0 Statistical Methods. • Section 12.2 was renamed to "Demographics". • MedDRA and WHO-Drug versions have been included. • Subgroup analysis have been added in Section 12.7 Efficacy Analyses • For the section and subsections 12.7.3 Assessment of Urinary Function, change from baseline is included for all timepoints (modified from the Protocol) • EPE and seminal vesicle invasion is excluded from section 12.7.7 Assessment of Ablation Effectiveness by Evaluation of Prostate Tissue by mpMRI, and tables. The definition for cancer-bearing prostate tissue lesions is included. • In section 12.7.8 Assessment of Need for Secondary or Adjuvant Treatment Following Treatment with the NanoKnife System, options "in-field or out-of-field" have been added to "positive unscheduled biopsy". TFLs have been also updated. • Section 12.8 Safety Analyses have been updated with clarity for re-treatment of lesions.

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	• Section 10.2 Adverse Events changing in severity/relationship with the treatment is created. • In section 12.8.3 Laboratory Data text have been modified to state that results will be categorical (normal, abnormal and not clinically significant, or abnormal and clinically significant). Laboratory tests have been specified. • Shift tables for urinalysis will be based on worst-case post-baseline value.
1.1/11-Apr-22 (SPONSOR & ICON APPROVED)	• In section 12.6 NanoKnife System Procedure, inclusion of "and device malfunctions (i.e., were there any device malfunctions or error messages during the procedure?)" • In section 12.7.10 Sensitivity Analysis, inclusion of a sensitivity analysis. • In Table 17, update laboratory tests and SI units, • In section 12.8.4 Vital Signs, adding BMI.
1.2/10-Mar-2023	• Imputation of Missing or Partial Dates • Updates after Protocol amendment – Protocol version 4.0 (date 20-Jan-2023) and CRF version 10.0 (date 14-Feb-2023): Inclusion/Exclusion criteria; possibility for combination of Screening and Baseline visit; Allow post-procedure visit to be conducted over the phone (if no urinalysis/MRI are needed)
2.0/06-Jun-2023 (SPONSOR & ICON APPROVED)	• Approved version after AngioDynamics' review of v 1.2

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3.0 Table of Contents

4.0 Purpose

The Statistical Analysis Plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under AngioDynamics, Protocol 2021-ONC-01.

5.0 Scope

The Statistical Analysis Plan outlines the following:

- Study Objectives
- Study Design
- Applicable Study Definitions
- Statistical Methods

6.0 Introduction

This SAP should be read in conjunction with the study protocol and case report forms (CRFs). Any further changes to the protocol or CRFs may necessitate updates to the SAP. This version of the plan has been developed using the protocol version 4.0 dated 20-Jan-2023 and CRF version 10.0 dated 14-Feb-2023.

This is a prospective study, with no blinding mechanism. A final version of the SAP will be issued for sponsor approval prior to database lock.

6.1 Changes from Protocol

There are no plans to further the follow-up beyond the 12 months. The continuation of follow-up for up to 5 years, as standard of care, to assess the rate of progression, re-intervention, and adverse events is not expected at this point. This exploratory endpoint stated in protocol is outside of the scope of this SAP.

No multiple imputation will be used for the missing items for 15-Item International Index of Erectile Function (IIEF-5), International Prostate Symptom Scores (IPSS), and Expanded Prostate Cancer Index Composite (EPIC) questionnaires, as stated in the protocol.

7.0 Study Objectives

7.1 Primary Study Objectives

- To determine the NanoKnife System's ablation effectiveness by measuring the negative in-field biopsy rate at 12 months.

- To determine the NanoKnife System's procedural and post-procedural safety profile by evaluating adverse event incidence, type, and severity through 12 months.

7.2 Secondary Study Objectives

- To evaluate urinary and erectile function after NanoKnife System treatment using validated subject questionnaires (University of California Los Angeles-EPIC[UCLA-EPIC] Urinary Domain [Wei et al., 2000], IPSS [Barry et al., 1992], and IPPS quality of life [IPSS-QoL] [Rees, 2013], IIEF-15 [Rosen et al., 2002]).
- To determine post-NanoKnife System treatment prostate-specific antigen (PSA) kinetics, including time to PSA nadir and post-nadir PSA stability.
- To determine the change in prostate volume by comparison of prostate volume measured on multiparametric magnetic resonance imaging (mpMRI) pre-treatment and at 12 months post-treatment.

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- To determine the effectiveness of therapy by assessing the need for secondary or adjuvant treatment following therapy.
- To determine health-related quality of life (HRQoL) levels after treatment with the NanoKnife System using a validated subject questionnaire – 5-dimension scale EuroQoL (EQ-5D) (Oppe et al., 2014).

8.0 Study Design

This is a prospective, non-randomized pilot study in 118 subjects treated at up to 20 clinical sites in the United States. The schedule of data collection is summarized below (Table 1).

This study will involve 118 subjects who meet the intermediate-risk criteria defined by this protocol. The biopsy and imaging techniques that will adopt within this trial are mpMRI and transperineal or transrectal prostate biopsy (fusion targeted biopsy of lesion plus 10-14 core systematic biopsies to include adequate sampling of peripheral zone). The subjects' prostate lesions, the locations of which will be determined by ultrasound-guided transperineal or transrectal prostate biopsy, will be targeted for treatment with the NanoKnife System. The primary objective of this the study will be to evaluate the NanoKnife System's ablation effectiveness by measuring the negative in-field biopsy rate at 12 months, and the procedural and post-treatment safety of the NanoKnife System via incidence and severity of adverse events through 12 months. The secondary objective of this study is to evaluate urinary and erectile function after NanoKnife System treatment using validated subject questionnaires, and to evaluate pre- and post-treatment changes in PSA and prostate volume. Other secondary outcomes include the effectiveness of the NanoKnife System by assessing the need for secondary or adjuvant treatment and health-related quality of life evaluated using validated subject questionnaires.

This group of subjects will be followed for safety and efficacy for 12 months. Safety will be assessed via incidence and severity of adverse events and evaluation of the treatment effect will be assessed via urologic function and quality of life. Local efficacy will be characterized via negative biopsy, post-treatment PSA kinetics including time to PSA nadir and post-nadir PSA stability, and changes in prostate volume.

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Table 1 Data Collection

Study Assessment	Visit 1	Visit 2	Visit 3	Post-Procedure[10]	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
	Screening	Baseline[3]	NanoKnife Treatment		1 month 30 ± 7 days	3 months 90 ± 14 days	6 months 180 ± 14 days	9 months 270 ± 14 days	12 months 365 ± 28 days
Informed Consent[1]	X								
Enrollment	X								
Medical History	X								
Standard Physical Exam		X			X	X	X	X	X
Abbreviated Physical Exam			X						
Vitals Assessment[4]		X	X		X	X	X	X	X
CBC Panel		X							
Prothrombin Test (PT)		X							
Partial Thromboplastin Time (PTT)		X							
Urinalysis		X	X[9]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
Transrectal US	X		X						X
MRI protocols	X[2]			X[6]		X			X
Biopsy[7,8]	X[2]								X
PSA	X[2]				X	X	X	X	X
UCLA-EPIC Urinary Questionnaire		X			X	X	X	X	X
IPSS & IPSS-QoL Questionnaire		X			X	X	X	X	X
IIEF-15 Potency Questionnaire		X			X	X	X	X	X
EQ-5D Questionnaire		X			X	X	X	X	X
NanoKnife System Procedure			X						
Concomitant Medications		X	X	X	X	X	X	X	X
Adverse Event Assessment			X	X	X	X	X	X	X

CBC = Complete blood count; EQ-5D = 5-Dimension scale EuroQoL; IIEF-15 = 15-Item international index of erectile function; IPSS = International prostate symptom scores; PSA = Prostate-Specific Antigen; QoL = Quality of life; UCLA-EPIC = UCLA expanded prostate cancer index composite; US = Ultrasound.

1. Must be signed and dated prior to completing any study procedures.
2. Must be completed within 180 days prior to enrollment in the study. Prostate-specific antigen for enrollment must be completed prior to biopsy, or must be taken more than 30 days after biopsy.
3. Complete within 30 days prior to NanoKnife System treatment.
4. Vitals assessments include height and weight, temperature, blood pressure, respiration rate and pulse. Note, height only needs to be collected at Baseline.
5. Complete only if there is a question of an active urinary tract infection.
6. Complete only if clinically indicated (i.e., the subject is experiencing unexpected adverse events following treatment).
7. Transperineal or Transrectal Prostate Biopsy (template mapping and/or limited targeted).
8. Complete at additional time points if subject has a lesion ≥ Prostate Imaging – Reporting and Data System 3 on the 3-month magnetic resonance imaging, experiences biochemical failure as defined by the Phoenix criteria as nadir + 2 ng/mL, or upon clinical suspicion.
9. Complete pre-treatment, and at discharge only if there is a question of an active UTI.
10. Complete within 3 to 10 days of NanoKnife System treatment. This visit can be conducted over the phone, as long as urinalysis and MRI are not clinically indicated.

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8.1 Sample Size Considerations

This study is designed to assess the efficacy of the NanoKnife System with respect to the primary efficacy endpoint (12-month negative in-field biopsy rate), as well as to assess the safety of the NanoKnife System with respect to the primary safety endpoint (rate of adverse events).

Assuming that 69% of the subjects undergoing treatment with the NanoKnife System will have an in-field negative 12-month biopsy, a minimum of 51 intent-to-treat (ITT) subjects are required to achieve at least 80% power to conclude that the NanoKnife System's in-field negative biopsy rate is at least 52% (as summarized in Table 2).

The performance goal of 52% was based on the Sonablate 450 de novo summary (DEN150011), which reported a negative biopsy rate of 61% at 12 months (missing data imputed as positive), minus a 9% non-inferiority margin.

Table 2 Sample Size Determination

Endpoint	Primary Analysis Population	Assumed Performance of the NanoKnife System	Performance Goal	Non-Inferiority Margin	Power	Sample Size
12-month negative in-field biopsy rate	ITT	0.69	0.52	0.09	80%	51

For safety, it was determined that there is greater than an 80% chance to detect at least one medically significant adverse event if the true event rate is at least 1.6% with a sample size of 100 subjects.

Allowing for a possible 15% drop-out rate for subjects who will be lost to follow-up, a minimum of 118 subjects will be enrolled in the clinical study.

8.2 Eligibility Criteria

Subjects will be considered enrolled in the study after consent has been obtained.

Subjects will be assessed for inclusion into this study using the criteria identified below. Subjects must meet all inclusion criteria and not meet any exclusion criteria in order to qualify for the study.

8.2.1 Inclusion Criteria

A subject is required to fulfill all of the following criteria to be included in the study:

1. Is greater than 50 years of age;
2. Has at least a 10-year life expectancy;
3. Has histologically confirmed organ-confined prostate cancer, clinical stage ≤ T2c;
4. Has a PSA ≤ 15 ng/mL or PSA density < 0.15 ng/mL^2 if PSA is > 15 ng/mL;
5. Has Gleason score 3+4 or 4+3;
6. Has no evidence of extraprostatic extension by mpMRI;
7. Has no evidence of seminal vesicle invasion by mpMRI, and if suspected, confirmed by biopsy;
8. Physician is able to visualize prostate gland adequately on transrectal ultrasound imaging during qualifying biopsy;
9. Has a transperineal or transrectal targeted prostate biopsy of lesion, plus 10-14 core systematic biopsy to include adequate sampling of the peripheral zone correlating with an intermediate risk lesion[1] in the area of the MR-visible lesion;

[1] An intermediate risk lesion is defined as Gleason score 3+4 or 4+3, PSA < 15 ng/mL or PSA density < 0.15 ng/mL^2 if PSA is > 15 ng/mL, and ≤ clinical stage T2c.

10. A visible lesion on mpMRI that is accessible to Irreversible Electroporation (IRE) treatment (Note: prostate cancer detected via systematic standard biopsy outside of the adjacent sextant location of the MRI visible lesion will meet entry criterion provided the positive core is Gleason 6; has fewer than 3 prostate biopsy fragments/cores positive and ≤50% cancer in each of those fragments/cores on standard biopsy);
11. Is willing and able to sign a written informed consent form and in the judgment of the physician, the study is in the best interest of the subject;
12. Understands and accepts the obligation and is logistically able to present for all scheduled follow-up visits.

8.2.2 Exclusion Criteria

A subject will be excluded from the study if they meet any of the following criteria:

1. Has known hypersensitivity to pancuronium bromide, atricurium or cisatricurium;
2. Is unfit for anesthesia or has a contraindication for agents listed for paralysis;
3. Has an active urinary tract infection (UTI);
4. Has a history of bladder neck contracture;
5. Is interested in future fertility;
6. Has a history (within 3 years) of inflammatory bowel disease;
7. Has a concurrent major debilitating illness;
8. Had active treatment for a malignancy within 3 years, including malignant melanoma, except for prostate cancer or other types of skin cancer;
9. Has any active implanted electronic device (e.g., pacemaker);
10. Is unable or unwilling to catheterize;
11. Has had any prior or current prostate cancer therapy including:
    a) Biologic therapy for prostate cancer;
    b) Chemotherapy for prostate cancer;
    c) Hormonal therapy for prostate cancer within three months of procedure;
    d) Radiotherapy for prostate cancer;
    e) Surgery for prostate cancer;
12. Has had prior transurethral prostatectomy (TURP), stricture surgery, urethral stent or prostatic implants;
13. Has had prior major rectal surgery (except hemorrhoids);
14. Is unfit for pelvic MRI scanning (e.g., severe claustrophobia, permanent cardiac pacemaker, metallic implants that are likely to contribute significant image artifacts, allergy or contraindication to gadolinium [to enhance MRI]);
15. Is actively bleeding, has a bleeding disorder, or is unable to interrupt blood thinning medications as clinically indicated per pre-operative best practices;
16. Is a member of a vulnerable population, such as cognitively impaired or incarcerated, that could expose them to undue influence, coercion, or inability to obtain informed consent;
17. In the opinion of the treating physician, has a contraindication listed in the current NanoKnife System User Manual (section 2.3).

9.0 Endpoints

9.1 Primary Endpoint

The primary treatment outcomes that will be evaluated are:

- Rate of negative in-field biopsy at 12 months – determine the rate of subjects obtaining a negative in-field biopsy on follow-up transperineal or transrectal biopsy at 12 months.
- Incidence of adverse events by type and Common Terminology Criteria for Adverse Events (CTCAE) v5.0 severity through 12 months – determine incidence, type, and severity during the NanoKnife System procedure and through follow-up.

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9.2 Secondary Endpoint

The secondary outcomes that will be evaluated are:

- Rate of negative in-field biopsy at 12 months as defined by the Delphi consensus criterion of absence of clinically significant disease (≤ 3 mm of Gleason ≤ 6 disease in any biopsy core is insignificant) (Postema et al., 2016).
- Assessment of urinary function by comparison of pre- and post-operative UCLA-EPIC Urinary Domain (Wei et al., 2000), IPSS (Barry et al., 1992), and IPSS-QoL scores (Rees, 2013).
- Assessment of erectile function by comparison of pre- and post-operative IIEF-15 potency scores (Rosen et al., 2002).
- Effectiveness of therapy by measurement of PSA kinetics including time to PSA nadir.
- Assessment of changes in prostate volume by comparison of pre-treatment and 12-month prostate volume measured via mpMRI.
- Assessment of ablation effectiveness by evaluation of prostate tissue by mpMRI at 3 months post-treatment and at 12 months post-treatment.
- Assessment of need for secondary or adjuvant treatment following treatment with the NanoKnife System.
- Evaluation of subject reported pre- and post-operative QoL using the EQ-5D® (Oppe et al, 2014).

9.3 Population Sets

9.3.1 Screening Set (SS)

The SS comprises all subjects screened that are captured in the EDC. This set will be used only to report subjects' analysis sets.

9.3.2 Intent-to-Treat (ITT) Population

The ITT population includes all subjects who were enrolled and treated with the NanoKnife System. Any subject who has a NanoKnife probe inserted will be considered treated for the ITT population. The ITT population will serve as the primary analysis set for all safety and efficacy endpoints.

9.3.3 Per protocol (PP) Population

The PP population includes all subjects who were enrolled and treated with the NanoKnife System, and who had no major protocol deviations. The PP population will serve as a supportive analysis set for all efficacy endpoints.

10.0 Conventions and Derivations

10.1 Baseline Definition

Baseline visit is considered the visit between Screening and NanoKnife System treatment visits. However, Screening and Baseline visits may be combined.

Baseline assessments will be completed for all subjects who have completed all screening assessments and meet all inclusion criteria and none of the exclusion criteria. At the time the subject presents for baseline, an assessment will be made to determine whether the subject still meets the Inclusion/Exclusion criteria.

These baseline assessments must be completed within 30 days of treatment with NanoKnife System.

10.2 Adverse Events changing in severity/relationship with the treatment

All AEs which change in severity or relationship to NanoKnife System treatment are assigned a new start date and captured as a new record.

10.3 Adverse Device Effects (ADEs) and Unanticipated Adverse Device Effects (UADE) Definition

Adverse device effects (ADEs) are a subset of adverse events (AEs). The ADEs are only those AEs caused by or related to the NanoKnife System. This definition includes any event resulting from insufficiencies or inadequacies in instructions for use, the deployment, or any malfunction of the device, including any event that is a result of a use error or intentional misuse.

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

10.4 PSA Nadir Definition

PSA nadir is the absolute lowest level that PSA achieves after the initial NanoKnife System procedure up until the time of re-treatment, if applicable.

10.5 Follow-up Visits and Visit Window

The following follow-up visit schedule and respective visit windows will be followed for each follow-up visit (Table 3).

Table 3 Visit Window

Visit	Visit Window
Post-Procedure[1]	3-10 days post-treatment
1 Month	30 ± 7 days
3 Month	90 ± 14 days
6 Month	180 ± 14 days
9 Month	270 ± 14 days
12 Month	365 ± 28 days

1. This visit can be conducted over the phone, as long as urinalysis and MRI are not clinically indicated.

10.6 Missing Data

Variables will be summarized for all eligible subjects with available data for the majority of endpoints/data (except for the endpoints/data detailed below). Missing data in the CRF will be detected by data management and monitored via edit checks and data analysis. Follow-up to collect missing data will be conducted as outlined in the Site Management Plan.

The following imputation methods will be applied:

- When calculating the proportion of subjects with a negative in-field biopsy, subjects with missing biopsy information post-ablation will be imputed as “positive”.
- Subjects with missing pre- or post-ablation prostate volume measurements will be imputed as having a zero change in volume. In accordance with the secondary endpoint related with “change in prostate volume”, this imputation will only be performed at Month 12 Visit.
- Missing PSA data will be imputed using last observation carried forward (LOCF).

10.6.1 Imputation of Missing or Partial Dates

Partial or missing dates will be imputed only for adverse events, concomitant medication, and other dates required for analysis (e.g., Time to PSA nadir [months] is calculated as [date of PSA nadir– NanoKnife System Treatment date + 1]/30.25). For the purpose of date imputation, Visit 8 (i.e., 12 Months) is defined as the last available visit date. E.g., for PSA nadir, subjects who are not observed to have encountered the event at end of study are censored at date of last observation. In listings original (non-imputed) dates will be presented.

The following rules will be applied to impute missing or partial dates in appropriate data types:

Parameter	Missing	Additional Conditions	Imputation
Start date	D	M and Y of the event date are the same as M and Y of first NanoKnife treatment	Day of first NanoKnife treatment
		Y of the event date is before Y of first NanoKnife treatment and M of the event date is before M of first NanoKnife treatment	Last day of the month
		Y of the event date is before Y of first NanoKnife treatment but M of the event date is after M of first NanoKnife treatment	First day of month
		Y of the event date is after or the same as Y of first NanoKnife treatment but M of the event date is before M of first NanoKnife treatment	Last day of the month
		Y of the event date is after or the same as Y of first NanoKnife treatment and M of the event date is after M of first NanoKnife treatment	First day of the month
		Patient is not treated	First day of the month
	D and M	Y of the event date is the same as Y of first NanoKnife treatment	Day and month of NanoKnife treatment
		Y of the event date is before Y of first NanoKnife treatment	31 Dec
		Y of the event date is after Y of first NanoKnife treatment	01 Jan
		Patient was not treated	01 Jan
	M only	If D of the event date is present and M of the event date is not present, D of the event date will be treated as missing	See imputation rule for 'D and M'
	D, M, Y	None - date completely missing	Date of first NanoKnife treatment
Stop date	D	M and Y of the event date are the same as M and Y of end of study	Day of End of study
		Y of the event date is before Y of end of study and M of the event date is before M of end of study	Last day of the month
		Y of the event date is before Y of end of study but M of the event date is after M of end of study	First day of month

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		Y of the event date is after or the same as Y of end of study but M of the event date is before M of end of study	Last day of the month
		Y of the event date is after or the same as Y of end of study and M of the event date is after M of end of study	First day of the month
	D and M	Y of the event date is same as Y of end of study	Day and Month of End of study
		Y of the event date is before Y of end of study	31 Dec
		Y of the event date is after Y of end of study	01 Jan
	M only	If D of the event date is present and M of the event date is not present, D of the event date will be treated as missing	See imputation rule for 'D and M'
	D, M, Y	None - date completely missing	No imputation, but assume ongoing
Note: In all cases, if an imputed start date is after a complete stop date, use the first day of the stop date month for start date. If stop date is before a complete or imputed start date, use either a) the last day of the start day month when start date is complete, or b) assign start date same as stop date when start date is imputed.			

11.0 Interim Analyses

No interim analysis is planned for this study.

12.0 Statistical Methods

All analyses will use SAS® version 9.4 or higher. Table and listing outputs will be presented using PRA standard layouts, but sponsor guidance for numbering of statistical outputs will be used.

Unless specified otherwise, categorical variables will be summarized using counts and percentages. Percentages will be rounded to one decimal place, except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts. Continuous variables will be summarized using the number of observations (n), mean, and standard deviation, median, minimum, 25th, and 75th percentiles, and maximum. The median, minimum, 25th, and 75th percentiles, and maximum values will be displayed to the same level of precision as the raw data, the mean and 95% confidence intervals (CI) to a further decimal place and the standard deviation to two additional decimal places to a maximum of four. The count of missing observations will be provided in all tables for information only.

The rate of negative in-field biopsy at 12 months will be calculated as the number of subjects with negative in-field biopsy on follow-up transperineal or transrectal biopsy at 12 months, divided by the total number of subjects still in the study at 12 months. Clopper-Pearson method (Clopper and Pearson, 1934) will be used to report 95% confidence interval (CI) as per requirement. The same approach will be followed for rate of negative in-field biopsy at 12 months as defined by the Delphi consensus criterion of absence of clinically significant disease (≤ 3 mm of Gleason ≤ 6 disease in any biopsy core is insignificant) (Postema et al., 2016).

For Kaplan-Meier method, the median time to event and associated 95% CI will be provided.
All statistical tests used generally are performed with a 2-sided alpha=5%. P-values will be rounded to 3 decimal places. P-values rounded to less than 0.001 will be reported as <0.001 in tables.

12.1 Subject Disposition

The number and percentage of subjects screened and included in each analysis set will be presented, together with the number and percentage of subjects who withdrew from the study prematurely and a

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breakdown of the corresponding reasons for withdrawal. A tabulation of the number and percentage of subjects enrolled at each center will be presented.

If a subject undergoes re-treatment, one re-treatment with IRE for the same lesion will allow the subject to continue in the study. However, if there is more than one re-treatment with IRE on the same lesion or at least one re-treatment with a type of treatment other than IRE, then the subject will exit the study (data until the moment of re-treatment will be used for analysis) and will only be followed up for safety purposes.

12.2 Demographics

Demographics will include: age at informed consent, race, and ethnicity. Demographics will be reported for both ITT and PP subjects.

12.2.1 Medical History

Information regarding medical history will be presented, for both ITT and PP subjects. The number and percentage of subjects with each medical history term will be summarized by the Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term. Medical history will be coded using MedDRA version 24.1 to assign a SOC and preferred term to each event.

12.3 Treatments

12.3.1 Prior Surgeries

Surgeries performed prior to NanoKnife will be reported for both ITT and PP subjects.

12.3.2 Concomitant Medications

Medications received concomitantly with study follow-up, during and after NanoKnife System treatment, categorized by medication group (ATC level 2) and subgroup (ATC level 4) according to WHODrug Sept2021), will be summarized. The number and percentage of subjects using each medication will be displayed together with the number and percentage of subjects using at least one medication within each medication group and subgroup. Concomitant medications will be reported for both ITT and PP subjects.

12.4 Prostate Cancer Diagnosis

Prostate Cancer Diagnosis information will be reported for both ITT and PP subjects. This information will comprise American Joint Committee on Cancer (AJCC) primary tumor stage, Gleason primary, secondary, and total sum score for the current diagnosis being treated.

12.5 Protocol Deviations

Per ICON Biotech Solutions processes, protocol deviations data will be entered into our system of record (Predictivv Study Operations [PSO]). The study team and the sponsor will conduct ongoing reviews of the deviation data from PSO and the resulting set of evaluable subjects throughout the study, adjusting the deviation criteria as seems appropriate. The evaluable subjects set must be finalized at the post-freeze data review meeting (or earlier), prior to database lock.

Based on the protocol deviations data entered into PSO, the protocol deviations thought to potentially impact the statistical analyses or subject safety will be listed and tabulated using incidence and percentages by deviation type for ITT subjects. Protocol deviations are defined in the protocol deviation guidance document. The last approved version of the protocol deviation guidance will be finalized before the database lock. The protocol deviance guidance will be used to exclude subjects from the PP population.

12.6 NanoKnife System Procedure

Details from the NanoKnife System procedure will be collected and presented in listings for both ITT and PP subjects. The details will comprise irreversible electroporation details, equipment used during NanoKnife procedure, physician assessment of procedure, and urinary catheterization. Device malfunctions will be summarized in a listing. Procedure duration (end time - start time), number of

electrodes, whether ultrasound/MRI fusion device was used during the procedure, urinary catheterization and its duration, physician assessment, and device malfunctions (i.e., were there any device malfunctions or error messages during the procedure?) will be also presented in a table for both ITT and PP subjects.

12.7 Efficacy Analyses

All efficacy analysis will be presented for the ITT population.

12.7.1 Rate of Subjects with a Negative In-field Biopsy at 12 Months

The number and proportion of subjects obtaining a negative in-field biopsy at 12 months will be presented, along with the 95% confidence interval of the proportion. Clopper-Pearson method will be used to report 95% CI.

Subjects who are biopsied prior to 12 months (if there are positive findings on the 3-month mpMRI, if the subject is deemed to have biochemical progression, or if the treating physician deems there to be clinical suspicion) and have a positive in-field biopsy will be imputed as failures for the analysis of the primary efficacy endpoint.[2]

The rate of negative in-field biopsies will be compared to a performance goal of 0.52 using a one-sample non-inferiority test for proportions.

The null and alternative hypotheses for negative biopsy rate at 12 months are as follows:

H_0: NanoKnife System ≤ (0.61 – margin of 0.09)

H_1: NanoKnife System > (0.61 – margin of 0.09).

For subjects with a positive biopsy, Gleason total score will also be detailed.

12.7.1.1 Subgroup analyses

Subgroup analyses will be performed for the primary efficacy endpoint as follows: i) Gleason score at screening: 3+4 vs 4+3; ii) Lesion size for the primary lesion (at visit 3): ≤ 3 mm vs > 3 mm; iii) Lesion location for the primary lesion (at visit 3): Base vs Apex; iv) Lesion location for the primary lesion (at visit 3): Anterior vs Posterior; v) PSA level at Screening: ≤ 15 ng/mL vs > 15 ng/mL.

12.7.2 Rate of Subjects with a Negative In-field Biopsy at 12 Months (Delphi Consensus)

The number and proportion of subjects obtaining a negative in-field biopsy at 12 months as defined by the Delphi consensus criterion of absence of clinically significant disease (≤ 3 mm in any biopsy core and Gleason Total Sum ≤ 6 disease in any biopsy core is insignificant [Postema et al., 2016]) will be presented, along with the 95% confidence interval of the proportion. Clopper-Pearson method will be used to report 95% CI.

For subjects with a positive biopsy, Gleason total score will also be detailed.

12.7.3 Assessment of Urinary Function

Urinary function is assessed by comparison of pre- and post-operative UCLA-EPIC Urinary Domain (Wei et al., 2000), IPSS (Barry et al., 1992), and IPSS-QoL scores (Rees, 2013).

Summary statistics will be presented for the timepoints where the questionnaires are collected (baseline, 1, 3, 6, 9, and 12 month follow-up). Additionally, all the follow-up changes from baseline will also be presented, along with the associated paired t-test value.

[2] Note: Subjects with early positive out-of-field biopsies will not be considered failures for the primary efficacy endpoint.

12.7.3.1 UCLA-EPIC Urinary Domain

For the purpose of assessing the urinary function, the UCLA-EPIC Urinary Domain will be used. This domain consists in 12 items and 4 subscales (Function [5 items], Bother [7 items], Incontinence [4 items], and Irritative/Obstructive [7 items]).

The response for each item is standardized from 0 to 100. The scores for the domain and subscales are the average of the standardized values. If ≥20% of the items of a domain score or subscale are missing a response, the corresponding domain summary or subscale cannot be calculated. For the Urinary Domain the minimum number of answers is 10, for Function subscale the minimum number of answers is 4, for the Bother subscale the minimum number of answers is 6, for the Incontinence subscale the minimum number of answers is 4, and for Irritative/Obstructive the minimum number of answers is 6.

For the Function subscale, the following questions are considered (Table 4):

Table 4 Function subscale questions

Question	Possible answers	Item Response Value	Standardized Value
Over the past 4 weeks, how often have you leaked urine?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
Over the past 4 weeks, how often have you urinated blood?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
Over the past 4 weeks, how often have you had pain or burning with urination?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
Which of the following best describes your urinary control during the last 4 weeks?	No urinary control whatsoever	1	0
	Frequent dribbling	2	33
	Occasional dribbling	3	67
	Total control	4	100
How many pads or adult diapers per day did you usually use to control leakage during the last 4 weeks?	None	0	100
	1 pad per day	1	67
	2 pads per day	2	33
	3 or more pads per day	3	0

For the Bother subscale, the following questions are considered (Table 5):

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Table 5 Bother subscale questions

Question	Possible answers	Item Response Value	Standardized Value
How big a problem, if any, has each of the following been for you during the last 4 weeks?			
Dripping or leaking urine	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Pain or burning on urination	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Bleeding with urination	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Week urine stream or incomplete emptying	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Waking up to urinate	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Need to urinate frequently during the day	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Overall, how big a problem has your urinary function been for you during the last 4 weeks?	No problem	1	100
	Very small problem	2	75
	Small problem	3	50

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Question	Possible answers	Item Response Value	Standardized Value
	Moderate problem	4	25
	Big problem	5	0

For the Incontinence subscale, the following questions are considered (Table 6):

Table 6 Incontinence subscale questions

Question	Possible answers	Item Response Value	Standardized Value
Over the past 4 weeks, how often have you leaked urine?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
Which of the following best describes your urinary control during the last 4 weeks?	No urinary control whatsoever	1	0
	Frequent dribbling	2	33
	Occasional dribbling	3	67
	Total control	4	100
How many pads or adult diapers per day did you usually use to control leakage during the last 4 weeks?	None	0	100
	1 pad per day	1	67
	2 pads per day	2	33
	3 or more pads per day	3	0
How big a problem, if any, has each of the following been for you during the last 4 weeks?			
Dripping or leaking urine	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0

For the Irritative/Obstructive subscale, the following questions are considered (Table 7):

Table 7 Irritative/Obstructive subscale questions

Question	Possible answers	Item Response Value	Standardized Value
Over the past 4 weeks, how often have you urinated blood?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75

Question	Possible answers	Item Response Value	Standardized Value
	Rarely or never	5	100
Over the past 4 weeks, how often have you had pain or burning with urination?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
How big a problem, if any, has each of the following been for you during the last 4 weeks?			
Pain or burning on urination	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Bleeding with urination	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Week urine stream or incomplete emptying	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Waking up to urinate	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Need to urinate frequently during the day	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0

For the Urinary Domain, all questions will be considered (without repeating them, see Table 8) (Wei et al., 2000).

Table 8 Urinary domain questions

Question	Possible answers	Item Response Value	Standardized Value
Over the past 4 weeks, how often have you leaked urine?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
Over the past 4 weeks, how often have you urinated blood?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
Over the past 4 weeks, how often have you had pain or burning with urination?	More than once a day	1	0
	About once a day	2	25
	More than once a week	3	50
	About once a week	4	75
	Rarely or never	5	100
Which of the following best describes your urinary control during the last 4 weeks?	No urinary control whatsoever	1	0
	Frequent dribbling	2	33
	Occasional dribbling	3	67
	Total control	4	100
How many pads or adult diapers per day did you usually use to control leakage during the last 4 weeks?	None	0	100
	1 pad per day	1	67
	2 pads per day	2	33
	3 or more pads per day	3	0
How big a problem, if any, has each of the following been for you during the last 4 weeks?			
Dripping or leaking urine	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Pain or burning on urination	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0

Question	Possible answers	Item Response Value	Standardized Value
Bleeding with urination	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Week urine stream or incomplete emptying	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Waking up to urinate	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Need to urinate frequently during the day	No problem	0	100
	Very small problem	1	75
	Small problem	2	50
	Moderate problem	3	25
	Big problem	4	0
Overall, how big a problem has your urinary function been for you during the last 4 weeks?	No problem	1	100
	Very small problem	2	75
	Small problem	3	50
	Moderate problem	4	25
	Big problem	5	0

12.7.3.2 IPSS

For the purpose of assessing the urinary function, the IPSS will be used. It consists of 8 questions, the first 7 related to severity of a range of urinary symptoms (three storage symptoms [urgency, frequency and nocturia] and four voiding symptoms [weak stream, hesitancy, intermittency and straining]) – scoring from 1 to 5 for each question to make a maximum total score of 35. Questions are detailed in Table 9, along with correspondent answers and respective item response value.

Table 9 IPSS questions

Question	Possible answers	Item Response Value
1.Over the past month, how often have you had a sensation of not emptying your bladder completely after you finished urinating?	Not at all	0
	Less than 1 time in 5	1
	Less than half the time	2
	About half the time	3
	More than half the time	4
	Almost always	5
2.Over the past month, how often have you had to urinate again less than 2 hours after you finished urinating?	Not at all	0
	Less than 1 time in 5	1
	Less than half the time	2
	About half the time	3
	More than half the time	4
	Almost always	5
3.Over the past month, how often have you found that you stopped and started again several times when you urinated?	Not at all	0
	Less than 1 time in 5	1
	Less than half the time	2
	About half the time	3
	More than half the time	4
	Almost always	5
4.Over the past month, how often have you found it difficult to postpone urination?	Not at all	0
	Less than 1 time in 5	1
	Less than half the time	2
	About half the time	3
	More than half the time	4
	Almost always	5
5.Over the past month, how often have you had a weak stream?	Not at all	0
	Less than 1 time in 5	1
	Less than half the time	2
	About half the time	3
	More than half the time	4
	Almost always	5
6.Over the past month, how often have you had to push or strain to begin urination?	Not at all	0
	Less than 1 time in 5	1
	Less than half the time	2
	About half the time	3
	More than half the time	4

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Question	Possible answers	Item Response Value
	Almost always	5
7. Over the past month or so, how many times did you get up to urinate from the time you went to bed until the time you got up in the morning?	None	0
	1 time	1
	2 times	2
	3 times	3
	4 times	4
	5 or more times	5

The total score will be the sum of all item scores. The total symptom score will be stratified as Mild (total score between 0 and 7), Moderate (from 8 to 19), and Severe (from 20 to 35). All items must be completed, no missing items are acceptable (Barry et al., 1992).

12.7.3.2.1 IPSS-QoL

For the purpose of assessing the urinary function, the IPSS-QoL will be used. It consists of a single question that assesses the impact of the symptoms detailed in IPSS on the subject's quality of life. Subjects select a score from zero (0) indicating delighted to six (6) indicating terrible (Rees, 2013), as detailed in Table 10.

Table 10 IPSS-QoL question

Question	Possible answers	Item Response Value
If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that?	Delighted	0
	Pleased	1
	Mostly satisfied	2
	Mixed about equally satisfied and dissatisfied	3
	Mostly dissatisfied	
	Unhappy	4
	Terrible	5
		6

12.7.4 Assessment of Erectile Function

For the purpose of assessing erectile function, the IIEF-15 questionnaire will be used. Summary statistics will be presented for the timepoints where the questionnaires are collected (baseline, 1, 3, 6, 9, and 12 month follow-up). Additionally, all the follow-up changes from baseline will also be presented, along with the associated paired t-test value.

The questionnaire consists of 15 questions, where 6 are related to erectile function, 3 refer to satisfaction with intercourse, 2 are related to orgasmic function, 2 refer to sexual desire, and 2 refer to overall sexual satisfaction.

The items are not weighted and total scores range from 5 to 75. The domain scores are computed by summing the scores for each individual item in each domain. The subscales range as follows: Erectile Function (1-30), Intercourse Satisfaction (0-15), Orgasmic Function (0-10), Sexual Desire (2-10), and Overall Sexual Satisfaction (2-10). On the Erectile Function subscale, lower scores indicate worse erectile

dysfunction, while on the remaining subscales, higher scores indicate less dysfunction. The overall score considers all questions. All items must be completed, no missing values are acceptable (Rosen et al., 2002).

For the Erectile Function subscale, the following questions are considered (Table 11):

Table 11 Erectile Function subscale questions

Question	Possible answers	Item Response Value
Over the last four weeks, how often were you able to get an erection during sexual activity?	No sexual activity	0
	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3
	Most times (more than half the time)	4
	Almost always or always	5
Over the last four weeks, when you had erections with sexual stimulation, how often were your erections hard enough for penetration?	No sexual activity	0
	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3
	Most times (more than half the time)	4
	Almost always or always	5
Over the last four weeks, when you attempted intercourse, how often were you able to penetrate (enter) your partner?	No sexual activity	0
	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3
	Most times (more than half the time)	4
	Almost always or always	5
Over the last four weeks, during sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	No sexual activity	0
	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3
	Most times (more than half the time)	4
	Almost always or always	5
Over the last four weeks, during sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	Did not attempt intercourse	0
	Extremely difficult	1
	Very difficult	2
	Difficult	3
	Slightly difficult	4
	Not difficult	5
Over the last four weeks, how do you rate your confidence that you can get and keep your erection?	Very low	1
	Low	2

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Question	Possible answers	Item Response Value
	Moderate	3
	High	4
	Very high	5

For the Intercourse Satisfaction subscale, the following questions are considered (Table 12):

Table 12 Intercourse Satisfaction subscale questions

Question	Possible answers	Item Response Value
Over the last four weeks, how many times have you attempted sexual intercourse?	No attempts	0
	1-2 times	1
	3-4 times	2
	5-6 times	3
	7-10 times	4
	11 or more times	5
Over the last four weeks, when you attempted sexual intercourse how often was it satisfactory for you?	Did not attempt intercourse	0
	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3
	Most times (more than half the time)	4
	Almost always or always	5
Over the last four weeks, how much have you enjoyed sexual intercourse?	No intercourse	0
	No enjoyment at all	1
	Not very enjoyable	2
	Fairly enjoyable	3
	Highly enjoyable	4
	Very highly enjoyable	5

For the Orgasmic Function subscale, the following questions are considered (Table 13):

Table 13 Orgasmic Function subscale questions

Question	Possible answers	Item Response Value
Over the last four weeks, when you had sexual stimulation or intercourse, how often did you ejaculate?	No sexual stimulation or intercourse	0
	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3

Question	Possible answers	Item Response Value
	Most times (more than half the time)	4
	Almost always or always	5
Over the last four weeks, when you had sexual stimulation or intercourse, how often did you have the feeling of orgasm or climax?	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3
	Most times (more than half the time)	4
	Almost always or always	5

For the Sexual Desire subscale, the following questions are considered (Table 14):

Table 14 Sexual Desire subscale questions

Question	Possible answers	Item Response Value
Over the last four weeks, how often have you felt sexual desire?	Almost never or never	1
	A few times (less than half the time)	2
	Sometimes (about half the time)	3
	Most times (more than half the time)	4
	Almost always or always	5
Over the last four weeks, how would you rate your level of sexual desire?	Very low or none at all	1
	Low	2
	Moderate	3
	High	4
	Very high	5

For the Overall Sexual Satisfaction subscale, the following questions are considered (Table 15):

Table 15 Overall Sexual Satisfaction subscale questions

Question	Possible answers	Item Response Value
Over the last four weeks, how satisfied have you been with your overall sex life?	Very dissatisfied	1
	Moderately dissatisfied	2
	Equally satisfied and dissatisfied	3
	Moderately satisfied	4
	Very satisfied	5
Over the last four weeks, how satisfied have you been with your sexual relationship with your partner?	Very dissatisfied	1
	Moderately dissatisfied	2
	Equally satisfied and dissatisfied	3

Question	Possible answers	Item Response Value
	Moderately satisfied	4
	Very satisfied	5

12.7.5 Effectiveness of Therapy by Measurement of PSA Kinetics

The mean PSA levels at each visit, the overall percent reduction in PSA levels (ng/mL) from screening to each visit (1, 3, 6, 9, and 12 month follow-up), and the proportion of subjects with a PSA reduction compared to screening at each visit will be presented. If a subject is re-treated during the study, all PSA values collected following the re-treatment will not be included in the analyses but will be included in the listings. Mean PSA nadir and post-nadir PSA values through 12 months post-treatment will be summarized. Kaplan-Meier analysis and summary statistics for time, including median time to event and associated 95% CI will be presented for PSA nadir. For this assessment, our baseline will be the screening measurements.

12.7.6 Assessment of Changes in Prostate Volume Measured via mpMRI

The mean prostate volume at each visit, the overall percent reduction in prostate volume from screening to 12 month follow-up, and the proportion of subjects with reduction in prostate volume compared to screening at 12 month follow-up will be presented, as measured by mpMRI. For this assessment, our baseline will be the screening measurements.

12.7.7 Assessment of Ablation Effectiveness by Evaluation of Prostate Tissue by mpMRI

The number and proportion of subjects determined to have cancer-bearing prostate tissue lesions (i.e., modified prostate imaging reporting and data system [PI-RADS] 3 or higher lesion) on the 3 and 12-month mpMRI will be presented.

12.7.8 Assessment of Need for Secondary or Adjuvant Treatment Following Treatment with the NanoKnife System

The number and proportion of subjects undergoing secondary or adjuvant treatment during the study will be presented. Additional information regarding reason (positive unscheduled biopsy [in-field or out-of-field], clinical progression, biochemical progression, or other), location (index lesion or contralateral lesion), and type of re-retreatment (re-treatment with IRE, radical prostatectomy, high-intensity focused ultrasound [HIFU], cryotherapy, or other) will also be presented.

12.7.9 Evaluation of Subject-Reported Quality of Life

For the purpose of assessing Quality of Life (QoL), the EQ-5D® will be used. Summary statistics will be presented for the timepoints where the questionnaire is collected (baseline, 1, 3, 6, 9, and 12 month follow-up).

The questionnaire has 5 dimensions from a descriptive system (Mobility, Self-care, Usual activities, Pain/discomfort, and Anxiety/depression), described in Table 16, and a visual analogue scale (VAS) related to subject's self-rated health.

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Table 16 EQ-5D dimensions on descriptive system

Dimensions	Possible answers	Item Response Level
Mobility	I have no problems in walking about	1
	I have slight problems in walking about	2
	I have moderate problems in walking about	3
	I have severe problems in walking about	4
	I am unable to walk about	5
Self-care	I have no problems washing or dressing myself	1
	I have slight problems washing or dressing myself	2
	I have moderate problems washing or dressing myself	3
	I have severe problems washing or dressing myself	4
	I am unable to wash or dress myself	5
Usual activities	I have no problems doing my usual activities	1
	I have slight problems doing my usual activities	2
	I have moderate problems doing my usual activities	3
	I have severe problems doing my usual activities	4
	I am unable to do my usual activities	5
Pain/discomfort	I have no pain or discomfort	1
	I have slight pain or discomfort	2
	I have moderate pain or discomfort	3
	I have severe pain or discomfort	4
	I have extreme pain or discomfort	5
Anxiety/depression	I am not anxious or depressed	1
	I am slightly anxious or depressed	2
	I am moderately anxious or depressed	3
	I am severely anxious or depressed	4
	I am extremely anxious or depressed	5

Each dimension answered will be associated with a level, from 1 to 5, as detailed in Table 16. The levels are the levels of perceived problems:

- Level 1: Indicating no problem;
- Level 2: Indicating slight problems;
- Level 3: Indicating moderate problems;
- Level 4: Indicating severe problems;
- Level 5: Indicating unable to/extreme problems.

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A unique health state is defined by combining one level from each of the five dimensions. It allows 3123 possible health states, where each state is referring to a 5-digit code. No total score will be presented for the EQ-5D. The unique health state for a subject will only be presented in a listing.

The EQ VAS asks how the subject would rate their health that day (i.e., "Your Health Today"). The answer is a quantitative value that ranges from 0 to 100, where 100 means best health possible the subject can imagine and 0 means the worst health the subject can imagine (Oppe at al., 2014, van Reenen et al., 2019). Summary statistics of the EQ VAS scorwill be presented for each timepoint. Additionally, change from baseline for all the follow-up timepoints of the EQ VAS scores will also be presented.

12.7.10 Sensitivity Analyses

All efficacy analysis will be repeated for the PP population. Additionally, a sensitivity analysis in the ITT population will be performed for the primary efficacy endpoint by only including subjects with biopsy data available at 12-months or who has a positive in-field biopsy prior to 12-months (i.e., without performing missing data imputation for subjects with missing biopsy information post-ablation).

12.8 Safety Analyses

All safety analyses will be presented for the ITT population. If a subject is re-treated with something other than the NanoKnife System during the study, the subject should exit the study; any safety data collected during and after the re-treatment will not be included in the safety analyses. Further, if a lesion is re-treated with the NanoKnife System more than once during the study, the subject should exit the study; any safety data collected for a subject after the second re-treatment of the same lesion will not be included in the safety analyses.

12.8.1 Adverse Events

All AEs reported by the sites will be recorded in the EDC. All AEs that meet the criteria for adjudication will be reviewed by the adjudication committee. For the events that are adjudicated, the adjudicated data will serve as the primary data source for analysis purposes. The site-reported AE data will be used for analysis of AEs that do not meet the criteria for adjudication.

A summary of all AEs will be presented, including the number of events and number and percentage of subjects reporting adverse events in the following categories: any AE (including 95% CI for the proportion), any severe AE, any ADE, any AE leading to death, any SAE, and any AE leading to study discontinuation. A

A breakdown of the number of events and the number and percentage of subjects reporting each AE, categorized by body system and preferred term coded according to the MedDRA dictionary, will be presented. Note that the counting of subjects with each event will be by subject rather than by event so that subjects are only counted once within each body system or preferred term.

A further tabulation of these data, categorized by relationship to NanoKnife System treatment, will be presented. When counting the number of subjects with each event, subjects with multiple events within a particular body system or preferred term will be counted under the category of their most procedure-related event within that body system or preferred term. Relationship to NanoKnife System treatment is categorized as: i) not related ("The cause of the AE is known and is not related to any aspect of study participation including the underlying condition"), , ii) reasonably possibly related ("There is a reasonable possibility that the event may have been caused by study participation. The AE has a timely relationship to the study procedure(s); however, it follows no known pattern of response and an alternative cause seems more likely or there is significant uncertainty"), iii) probably related ("It is probable that the event was caused by study participation. The AE has a timely relationship to the study procedure(s) and follows a known pattern of response, but a potential alternative cause may be present."), iv) definitely related ("The event was definitely related to study participation. A related event has a strong temporal relationship and an alternative cause is unlikely.") , or v) unknown as recorded on the CRF.

A summary of events reported, categorized by severity (Mild [Grade 1], Moderate [Grade 2], Severe or medically significant but not immediately life-threatening [Grade 3], Life-threatening consequences [Grade

4], and Death [Grade 5]), will also be provided. Grades are determined according to CTCAE severity grades. When counting the number of subjects with each event, subjects with multiple events within a particular body system or preferred term will be counted under the category of their most severe event within that body system or preferred term.

All AEs recorded on the CRF will be provided in a listing. Independent listings will be provided with MedDRA system organ class (SOC), preferred and Verbatim terms for AEs, AEs with outcome of death, AEs leading to study discontinuation, and AEs related to NanoKnife treatment leading to study discontinuation.

12.8.2 Deaths and Serious Adverse Events

Serious adverse events (SAEs) and NanoKnife System treatment-related SAEs will be summarized separately by body system and preferred term for the ITT subjects. All SAEs recorded on the CRF will be provided in a listing for subjects in the ITT population.

A table presenting the number of events and the number and percentage of subjects by body system and preferred term who experienced SAEs related to NanoKnife System treatment during the study will be presented for the ITT population. UADEs and deaths occurring in the study will also be listed for all subjects in the ITT population.

12.8.3 Laboratory Data

Blood samples will be drawn for complete blood count (CBC) and for coagulation tests (Table 17). Hematology and coagulation values are only collected at baseline. Laboratory baseline values for blood samples will be summarized using descriptive statistics for all subjects in the ITT population-

Table 17 Laboratory Tests for Hematology and Coagulation Parameters

Laboratory test	SI Units	Laboratory test	SI Units
Red Blood Cells (RBC, erythrocytes)	10^{12}/L	Neutrophils (Differential)	%
Hematocrit	fraction of 1	Lymphocytes (Differential)	%
Hemoglobin	g/L	Monocytes (Differential)	%
Erythrocytes MCH	pg	Eosinophils (Differential)	%
Erythrocytes MCHC	g/L	Basophils (Differential)	%
Erythrocytes MCV	fL	Activated Partial Thromboplastin Time (aPTT)	s
White Blood Cells (WBC, leukocytes)	10^{9}/L	Prothrombin Time (PT)	s
Platelets	10^{9}/L	Prothrombin International Normalized Ratio (INR)	a

a, adimensional; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; SI, International System of Units.

Urine samples will be collected at baseline and during study follow-up if there is any question of an active urinary tract infection.

Laboratory tests for urinalysis will be: Specimen Appearance, Color, Specific Gravity, pH, Protein, Glucose, Occult Blood, Ketones, Nitrate, Bilirubin, Leukocyte Esterase, Microscopic Findings: RBC, Microscopic Findings: WBC, Microscopic Findings: Bacteria/Yeast, Microscopic Findings: Casts, Microscopic Findings: Epithelial Cells, Microscopic Findings: Crystals. Laboratory values for urinalysis will be summarized using the frequencies (number and %) of subjects with normal, abnormal but not clinically significant, and abnormal and clinically significant at each timepoint.

Shift tables of the worst-case post-baseline value will be presented by reference range (i.e., normal, abnormal but not clinically significant, and abnormal and clinically significant) for urinalysis. Shift tables will include all urinalysis assessments.

12.8.4 Vital Signs

Values and change from baseline in vital signs will be summarized by time point for all subjects in the ITT population. Descriptive statistics will be presented. Parameters include height (only at baseline, in cm),

weight (kg), body mass index (BMI, kg/m^2), temperature (ºC), systolic and diastolic blood pressure (mmHg), respiration rate (breaths/min), and pulse (beats/min).

12.8.5 Physical Examinations

Results of physical examinations will be summarized by time point for all subjects in the ITT population. A tabulation of the number and percentage with each possible result will be presented for applicable body systems. Parameters include a standard physical exam by body system to assess if all is normal, and if not if clinically significant.

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14.0 Glossary of Abbreviations

Glossary of Abbreviations:	
ADE	Adverse Device Effects
AE	Adverse Event
AJCC	American Joint Committee on Cancer
aPTT	activated Partial Thromboplastin Time
ATC	Anatomic Therapeutic Classification
BMI	Body Mass Index
CBC	Complete Blood Count
CI	Confidence Interval
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
eCRF	electronic Case Report Form
EQ-5D	5-Dimension scale EuroQoL
EPE	ExtraProstatic Extension
ESUR	European Society of Urogenital Radiology
HIFU	High-Intensity Focused Ultrasound
HRQoL	Health-Related Quality of Life
IIEF-15	15-item International Index of Erectile Function
INR	International Normalized Ratio (of Prothrombin)
IPSS	International Prostate Symptom Scores
IRE	IRreversible Electroporation
ITT	Intention-To-Treat
LOCF	Last Observation Carried Forward
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mpMRI	multiparametric Magnetic Resonance Imaging
MRI	Magnetic Resonance Imaging
PI-RADS	Prostate Imaging-Reporting And Data System
PP	Per Protocol
PSA	Prostate-Specific Antigen
PSO	Predictivv Study Operations
PT	Prothrombin Time

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QoL	Quality of Life
RBC	Red Blood Cells
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SI	International System of Units
SOC	System Organ Class
SS	Screening Set
TURP	TransURethral Prostatectomy
UADE	Unanticipated adverse device effect
UCLA-EPIC	University of California Los Angeles-Expanded Prostate cancer Index Composite
UTI	Urinary Tract Infection
VAS	Visual Analogue Scale
WBC	White Blood Cells
WHO-Drug	World Health Organization drug dictionary

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