USC Norris Comprehensive Cancer Center
Part of the Keck School of Medicine of USC

Local Protocol #: 4P-23-1

Protocol Title: MRI/Ultrasound fusion guided Transperineal Targeted Microwave Ablation for Prostate Cancer

Protocol version date: 6/16/2023

ClinicalTrialsgov #: NCT06128525

UNIVERSITY OF SOUTHERN CALIFORNIA/KENNETH NORRIS, JR. COMPREHENSIVE CANCER CENTER AND HOSPITAL

PRAMA (Prostate Resection After Microwave Ablation)

MRI/Ultrasound fusion guided Transperineal Targeted Microwave Ablation for Prostate Cancer

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Study Drug: NA

IND Number: NA

Funding Source: Funding from Koelis (Medical device company)

Initial version: 06/16/2023
Amended:

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Signature Page

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The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name: **Andre Luis De Castro Abreu**

PI Signature: _____

Institutional Name: USC Institute of Urology

Date: _____

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LIST OF ABBREVIATIONS

ADC	apparent diffusion coefficient
ADT	androgen deprivation therapy
AE	adverse event
AS	active surveillance
BP	blood pressure
CDC	Clavien Dindo classification
CEUS	contrast enhanced ultrasonography
CISO	Clinical Investigations Support Office
CRO	Clinical Research Organization
DCE	dynamic contrast enhanced
DSMC	Data and Safety Monitoring Committee
DWI	diffusion weighted images
eCRF	Electronic Case Report Form
EPIC	Expanded Prostate Cancer Index Composite
FDA	The U.S. Food and Drug Administration
FT	focal therapy
GCP	Good Clinical Practice
H&E	hematoxylin and eosin
HIFU	high intensity focused ultrasound
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICU	intensive care unit
IIEF	International Index of Erectile Function
IND	Investigational New Drug Application
IPSS	International Prostate Symptom Score
IRB	institutional review board
ISUP	International Society of Urological Pathology
IV	intravenous
mpMRI	multiparametric magnetic resonance imaging
MRI	magnetic resonance imaging
NA	not applicable
NADH	nicotinamide adenine dinucleotide hydrogen
NADPH	nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
OR	operation room
PCa	prostate cancer
PIRADS	Prostate Imaging Reporting & Data System
PRAMA	Prostate Resection After Microwave Ablation
PSA	prostate specific antigen
ROI	region of interest
RP	Radical prostatectomy
SAE	Serious Adverse Event
TMA	Targeted Microwave Ablation

TRUS	transrectal ultrasonography
TTC	Triphenyltetrazolium chloride
US	Ultrasound
USC	University of Southern California

STUDY SCHEMA

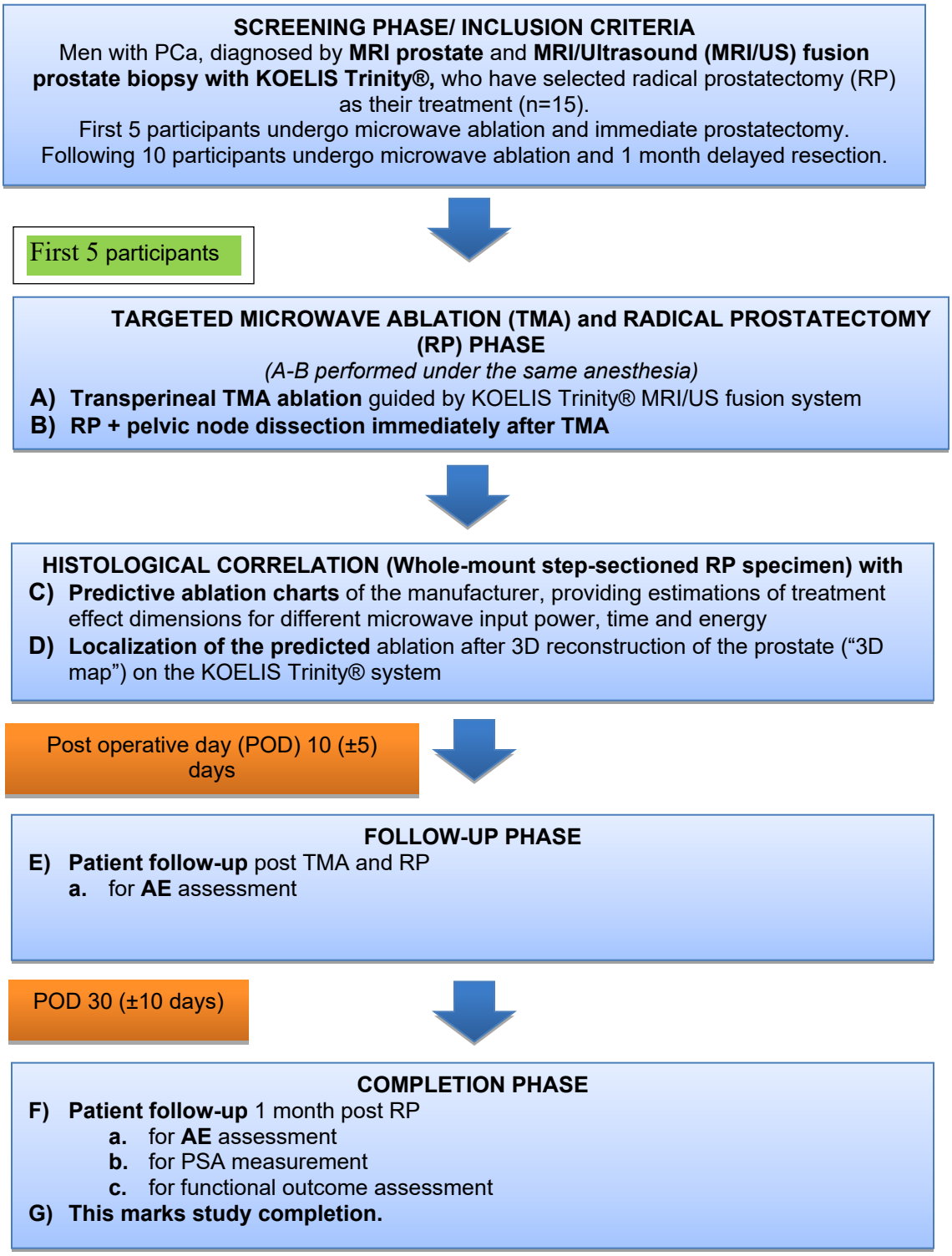
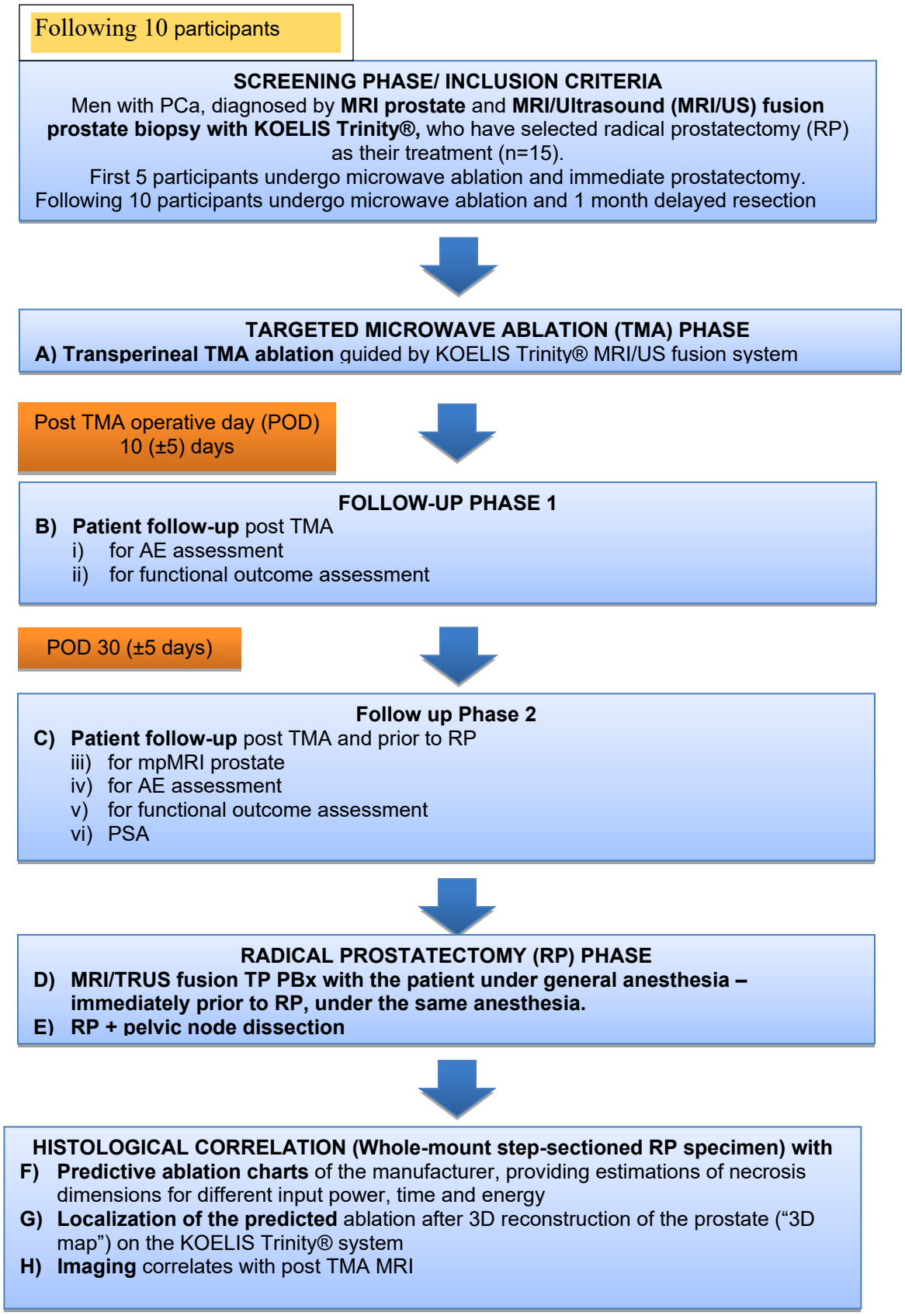


Figure 1. Study schema for immediate resection part

STUDY SCHEMA continued



STUDY SCHEMA continued

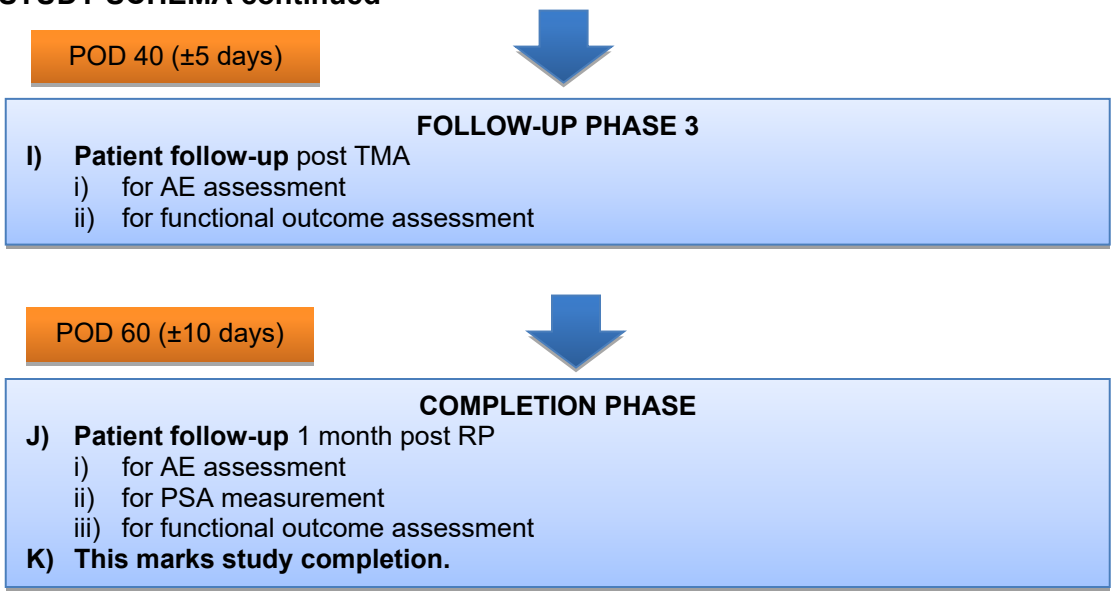


Figure 2. Study schema for delayed resection part

STUDY SUMMARY

Title	PRAMA (Prostate Resection After Microwave Ablation) MRI/Ultrasound fusion guided Transperineal Targeted Microwave Ablation for Prostate Cancer
Short Title	PRAMA (Prostate Resection After Microwave Ablation)
Protocol Number	
Phase	Phase I
Methodology	Clinical Trial
Study Duration	One and a half year
Study Center(s)	USC Institute of Urology
Primary Objective and related endpoint	**- Feasibility of transperineal targeted microwave ablation (TMA) of an MRI-identified index prostate tumor in patients with prostate cancer and eligible for radical prostatectomy.** ➔ The ablated area on the radical prostatectomy specimen assessed by viability tissue staining (TTC).

Secondary Objectives and related endpoints	**- KOELIS Trinity ability to safely plan and guide treatment needles and ablations in the prostate index lesion** ➔ Number and severity of device- and procedure-related adverse events ➔ TMA procedure duration: probe-in probe-out time, ablation time ➔ Comparison between the ablation location in the prostate as seen in the KOELIS 3D reconstruction of the prostate ("3D Map") and the ablation location observed histologically on the prostatectomy specimen **- Impact of treatments on functional outcomes and quality of life** ➔ Change in urinary functions between baseline and each follow-up visit, using IPSS score and uroflowmetry ➔ Change in erectile functions between baseline and each follow-up visit, using IIEF-5 score Change in quality of life between baseline and each follow-up visit, using EPIC-26 score **- Predictability of Medwaves Avecure microwave ablation charts** ➔ Correlation between the treatment effect dimensions measured histologically on the prostatectomy specimen resected immediately or 1mo after TMA, compared to the predictive ablation charts provided by the manufacturer. ➔ Evaluation of the treatment parameters to induce the cell destruction without causing collateral damage **- Predictability of post microwave ablation mpMRI** ➔ Correlation between the treatment effect dimensions measured histologically on the prostatectomy specimen resected 1 mo after TMA, compared to the post ablation dimensions measured on the mpMRI done prior to RP.
Number of Subjects	15 men with possible extension to more interventions

Diagnosis and Main Inclusion Criteria	• Male • Index lesion visible on multiparametric MRI confirmed by targeted transperineal biopsies using KOELIS Trinity® • Having a diagnosis of prostate cancer (Gleason score ≤8) • Prostate size ≤ 150 cc • Patient suitable for IV sedation or general anesthesia and focal targeted microwave ablation • Having elected to undergo RP as treatment of choice • Ability to understand and the willingness to sign a written informed consent • Free, informed, and written consent, dated and signed before the enrollment and before any exam required by the trial
Study Product(s), Dose, Route, Regimen	A TMA ablation will be performed during each intervention.
Duration of administration	NA
Reference therapy	NA
Statistical Methodology	For practical considerations, the expected total number of study participants is 15 patients. This number may be extended in case further ablation parameters need to be tested. An analysis is planned to determine TMA delivery protocol (number of ablations, energy output, and time needed to ablate one MRI-visible lesion). We will assess the correlation between the treatment effect dimensions measured histologically on the prostatectomy specimen resected immediately or 1mo after targeted microwave ablation, compared to the predictive ablation charts provided by the manufacturer. We will report theablated area on the radical prostatectomy specimen assessed by viability tissue staining (TTC) on all fully evaluable patients.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

Prostate cancer (PCa) is the second most common cancer in men, with approximately 1,400,000 men diagnosed and 375,000 who died of it worldwide in 2020 [1]. Most diagnosed Pca are localized and will never become aggressive during a patient's lifetime.

A great proportion of these men would benefit from enrolling in active surveillance (AS), which consists of monitoring with repeated biopsy and follow-up, and forgo any definitive treatments. Although AS has oncologic outcomes comparable to those of definitive treatments for low-risk disease patients [2]–[9], only ~40% of patients elect this modality due to perceived uncertainty about the aggressiveness of the tumor [10][11]. Further, within 5 years of initiating AS, over 35% of those who choose this starting therapy discontinue it in favor of definitive treatment. Therefore, most men with localized Pca still overwhelmingly elect to undergo treatment, whether it is radical prostatectomy (RP) surgery, radiation or androgen deprivation therapy (ADT), which can have a significant negative impact on their quality of life [12]–[14].

Therefore, there is a great need for minimally invasive focal therapies (FT) that might allow patients to achieve the same benefits as of whole-gland therapies (RP, radiation) while avoiding the quality-of-life consequences of these definitive treatments. In order for these focal therapies to be successful, For focal therapy to succeed, requirements are an accurate localization of its geographic location, preferably with data-recall capability, and precise delivery of a cytocidal energy under image guidance [15]

Moreover, non-invasive approaches to monitor disease eradication post-treatment need to be validated as well. In the recent past, several technologies and approaches have been developed that offer the possibility to do focal treatment of the prostate. In this study, we are focused on validating the use of targeted microwave ablation (TMA) for focal guided and targeted ablation of prostate cancer.

1.2 The use of TMA (Study Agent): opportunities and challenges

FT including High Intensity Focused Ultrasound (HIFU) or cryosurgery have emerged as promising approaches that could achieve similar oncological results compared with whole-gland treatments, with the benefit of reduced morbidity and superior functional outcomes.

Of particular concern are reports of the substantial rate of 'in-field' positive biopsies after "technically-successful" post FT (up to 12-35%) [14],[16]–[21]. Moreover, a small study reported a higher than expected number of viable cancer cells in post-FT RP specimens. Thus, there is an unmet need with optimal targeting and ablation capabilities.

Microwave ablation is one of the ablation therapies that induces cell death by damaging cellular membrane or intracellular-structure membrane, as well as by denaturing and coagulating structural proteins and eradicating the local blood flow. This ablation method is used as a curative treatment option for renal cancers, liver cancers, and lung cancers [22]–[25]. So far, only a few studies investigated targeted microwave ablation for Pca

[26][27][28]. However, there is no study evaluating TMA area with human RP specimen histology. Therefore, the optimal TMA delivery protocol has not been well investigated.

When performing focal thermal ablation therapy such as cryoablation and HIFU, high blood flow adjacent to the ablated area may prevent effective ablation [29]–[31]. It is known as heat-sink effect. Previous studies reported microwave can rapidly generate high energy and ablate tissues in less time with limited heat-sink effect, and the volume of the microwave area can be predictable and repeatable [32],[33]. Compared to existing ablative technologies, the main advantages of microwave include higher intra-tumoral temperatures, larger tumor ablation volumes, faster ablation time, better intraoperative visualization and treatment monitoring, an improved convection profile, and less procedural pain [33].

Targeting of the microwave to a given anatomic location within the prostate is currently possible given the advances in real-time MRI-TRUS image-fusion technology, which allows “highly accurate” tissue targeting and ablation. Although HIFU is one of the FDA-approved focal ablation treatment technology for prostate cancer and performed worldwide, TMA has several advantages over HIFU. TMA is easier to position, since the TMA antenna is directly placed in the tumor and moves with tumor, even if the tumor shrinks with the ablative treatment. In addition, while HIFU treatment plans must avoid treatment beams passing through critical structures, such as urethra or prostatic calcifications, TMA can freely target the cancerous lesions even located in anterior of these critical structures [33].

Surgical planning using 3D ultrasound and real-time monitoring of the ablation would thus preserve key anatomical landmarks. The Organ-Based Tracking (OBT®) patented technology would provide a 3D prostate model to facilitate real-time navigation-guided ablation and accurate mapping of the treatment zones, as well as automatically recalibrating the MRI/US fusion throughout the exam.

Therefore, we believe TMA can be a treatment option for MRI-TRUS fusion biopsy proven Pca.

1.3 Rationale for this study

Optimal FT modality to balance prostate cancer control and functional preservation is needed. However, current FT shows the substantial rate of recurrence from the ablated area. It may be partially due to the heat-sink effect. The existing literature and our own clinical experience with patients failing FT and subsequently undergoing salvage robotic RP has raised real-life concerns of the clear possibility of inaccurate targeting and inadequate ablation with current FT modalities, leaving behind residual cancer cells [16]–[21]. This problem is compounded by the fact that there are no studies that report on the validity of using existing tools to monitor FT success after treatment or shortly after, other than monitoring PSA and doing repeat biopsies. Therefore, current FT-treated patients may go many months without knowing their treatment has failed.

Microwave is known to be able to ablate tissues immediately and homogeneously with limited heat-sink effect and short ablation times based on previous studies for other organs [32],[33]. Therefore, microwave is a promising energy source for Pca treatment.

TMA for Pca has been studied in the recent years and has shown an excellent safety profile and promising oncological outcomes[26][27][28]. However, to date no study has been performed to evaluate TMA with radical prostatectomy specimen histology immediate post-ablative resection. .
To establish TMA as effective FT option for Pca from oncologic and functional perspective, optimal TMA protocol should be identified.

Important unanswered questions about the feasibility and efficacy of TMA include:

1) Does an TMA-intended lesion accurately mirror the actual histologically-confirmed tissue destruction in man?
2) Are there skip-lesions of viable tissue within a 'well-created' TMA lesion?
3) What is the optimal TMA protocol (i.e. ablation sessions, output energy, and ablation time) to acquire sufficient tissue ablation?
4) How safe is TMA intra/post operatively?
5) Can multiparametric MRI as contemporary imaging modality accurately confirm the completeness of TMA-induced Prostate tissue treatment effect at the histologic level?

Definitive data attesting to the precision of TMA at the Pca tissue level are critical, as well as data documenting the most adequate treatment parameters to achieve such precision. Such fundamental, prostate tissue-level data in the human are lacking. To date, no published human studies have reported spatial thoroughness of TMA prostate tissue kill as correlated with contemporary imaging and histology to assess and predict tissue destruction; nor have TMA technologic parameters been directly correlated with thoroughness of human prostate tissue kill.

The critical barrier to solve this problem is the ability to thoroughly examine the whole prostate gland in relatively close proximity to TMA ablation, timed appropriately to measure the relevant changes induced. Analyses should be soon enough before the ablated tissue starts to undergo fibrotic atrophy and shrinkage, as these would invalidate accurate comparison between imaging versus histologic measurements (e.g. salvage RP many months after TMA would not accomplish this, and it would also result in worse clinical outcomes for the patient due to peri-prostatic scarring involving the neurovascular bundles and sphincter).

A Method, named as Triphenyltetrazolium chloride (TTC) staining, for assessing lethal thermal tissue injury is available which rely on the functional status of cellular enzymes and mitochondrial energy production to produce differential staining between thermal tissue necrosis and adjacent untreated viable tissue. The dehydrogenase enzymes assessed by this staining method are essentially within the mitochondria and utilize NADH/NADPH as electron donors to promote redox reactions. This viability stains use members of the tetrazolium family as their chromogen (stain colorant). The dehydrogenase enzymes and their cofactors (NADH and NADPH) reduce the colorless tetrazolium molecule into a colored formazan pigment (viable tissue). Following a thermal treatment, tissues with resultant denatured cellular enzymes and/or disrupted mitochondrial cofactor production will be unable to convert tetrazolium to its colored form and remain their native color (non-viable tissue) [34]. We adapt TTC staining to assess the location and extent of a treatment's ablation/thermal effect within the prostate. The use of TTC staining does not limit subsequent histologic prostate evaluation which is essential for optimal patient standard of care.

When performing and evaluating viability stains, timing of the staining procedure is important. On the other hand, TTC staining should not be performed less than 2 hours from completion of the hyperthermic ablation. This post-treatment period allows for any residual enzyme and cofactor activity to fully cease and limits the risk of false positive staining. Thus, TTC staining should be performed more than 2 hours following the ablation and within 6 hours from tissue excision from the patient. This strict time frame is a rationale of the current ablation and immediate resect study protocol.

In order to evaluate the feasibility of TMA in more practical setting, further analyses should be delayed long enough post-TMA treatment to allow tissue changes of irreversible thermal damage to set in. In this setting, we evaluate the ablation effect on histology using HE staining instead of TTC staining. This delayed resection allows us to evaluate short term safety of TMA and predictivity of post ablation mpMRI. It may also allow us to identify potential histological elements occurring after TMA, such as fibrosis or scar tissue.

In this way, we will safely evaluate the optimal TMA protocol based on the first 5 cases, and will evaluate the practical feasibility of TMA using the following 10 cases.

We do not anticipate pre-surgical TMA will cause any significant technical difficulties during the performance of RP. Ablate and early resect studies have been performed after focal prostate ablation (HIFU, electroporation) with no reporting of adverse patient outcomes [35],[36]. Additionally, salvage (delayed) radical prostatectomy, years after failure of whole gland ablation, which are arguably much more challenging, is not an uncommon procedure offered at many centers [37],[38]. In fact here at USC, we have safely performed many (>100) salvage prostatectomies.

Our proposed, prospective, “ablate & resect” trial will uniquely solve this problem while addressing the existing barriers. Recent advances in Pca imaging and targeted biopsy allow us to comprehensively address this problem. By definitively answering each of the above-listed five questions, our proposal will add novel scientific knowledge about tissue-level effects of prostate TMA in man; will for the first time corroborate these data with contemporary imaging and biopsy; will potentially improve the technical capability and delivery of TMA for Pca application; and thereby impact clinical practice for men desiring treatment for non-metastatic Pca.

If successful, our study will have important clinical implications. It will definitively document whether TMA creates clinically reproducible targeted, irreversible destruction of prostate tissue in the human. This will be confirmed with the gold-standard of step-section RP histology and TTC staining. Such tissue-level data is the first of its kind in the field. If we are successful, a multicenter prospective study could be implemented to further validate our findings.

1.4 Goal of Proposed Study

Our overall goal is to assess the feasibility of the TMA procedure by measuring its efficacy with the gold standard method (radical prostatectomy histological evaluation), its precision using the MRI/US guidance with organ-based tracking technology, and its safety with procedure- and device-related adverse events.

1.5 Hypothesis

There are two main hypotheses:
1) The microwave energy can safely kill prostate cancer cells in the treated area, eradicating evidence of prostate cancer
2) The use of MRI/US image-fusion targeted biopsies technique with OBT-Fusion® allows a safe and precise guidance of a therapeutic needle in the area to be treated that was determined pre-operatively.

1.6 Study Overview

We propose to conduct a comprehensive single institution 'ablate and resect' trial on Targeted Microwave Ablation (TMA) in patients undergoing robotic RP. Briefly, we will recruit patients with localized Pca undergoing RP as their treatment of choice. Immediately or 1 mo before performing RP, we will ablate the prostate with focal TMA. TMA treatment parameters will be chosen according to the manufacturer data and will be visualized in 3D using an MRI/US image-fusion platform (KOELIS Trinity®). After the prostatectomy we will study the entire prostate so that we can determine the rate of TMA-induced cell death. We will use a histologic step-sectioning approach to process the prostate that will allow us to correlate 1:1 the histology with imaging. The results of our study will provide answers to the following important questions.

Important question #1: Is TMA effective in eradicating all prostatic tissue and prostate cancer cells within the ablated area?
Important question #2: Can TMA be safely and precisely delivered to an index lesion of the prostate using the KOELIS Trinity® platform?
Important question #3: Do TMA predictive treated areas dimensions correlate with ablated area as determined by histological changes measured via TTC staining post-TMA on the prostatectomy specimen?
Important question #4: What are the best TMA parameters to achieve maximum cell kill without causing collateral complications?
Important question #5: How accurately post TMA MRI predict the treated area's dimensions correlate with ablated area as determined by histological changes.
Important question #6: What are the MRI findings after TMA and how the MRI correlates to histology on prostate biopsy and RP specimen?
Important question #7: What is the perioperative AE after TMA?
Important question #8: What is the feasibility and safety of RP after TMA in case of ablation failure?

A total of 15 patients will be enrolled who will be identified from patients who have elected to undergo RP as their definitive treatment for PCa.
Immediately prior to RP surgery, 5 patients will undergo TMA with one ablation in the prostate index lesion.
One month prior to RP surgery, following 10 patients will undergo TMA with one ablation in the prostate index lesion.
The number of patients included may be extended considering the need to evaluate different treatment parameters (still according to manufacturer dat.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

To determine the feasibility of transperineal targeted microwave ablation of an MRI-identified index prostate cancer in patients undergoing radical prostatectomy.

2.2 Secondary Objectives

- KOELIS Trinity ability to plan and guide treatment needles and ablations in the prostate index lesion
- Impact of treatments on functional outcomes and quality of life
- Predictability of Medwaves Avecure microwave ablation charts
- Predictability of post microwave ablation mpMRI

2.3 Descriptive Objectives

NA

2.4 Primary Endpoints

The ablated area on the radical prostatectomy specimen assessed by viability tissue staining (TTC).

2.5 Secondary Endpoints

- The intra/post-operative safety of the targeted microwave ablation (number and severity of device- and procedure-related adverse events,
- TMA procedure duration: probe-in / probe-out time, ablation time
- Comparison between the ablation location in the prostate as seen in the KOELIS 3D map and the ablation location observed histologically on the prostatectomy specimen
- The change in urinary functions between baseline and each follow-up visit, using IPSS score and uroflowmetry
- The change in erectile functions between baseline and each follow-up visit, using IIEF-5 score
- The change in quality of life between baseline and each follow-up visit, using EPIC-26 score
- The correlation between the treatment effect dimensions measured histologically on the prostatectomy specimen resected after TMA, compared to the predictive ablation charts provided by the manufacturer
- The correlation between the treatment effect dimensions measured histologically on the prostatectomy specimen resected one month after TMA, compared to post TMA dimensions measured on the pre-RP mpMRI.
- Evaluation of the optimal treatment parameters to maximize the cell destruction without causing collateral damage.

2.6 Descriptive Endpoints

NA

3.0 PATIENT ELIGIBILITY

Eligibility waivers are not permitted. Subjects must meet the inclusion and exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

3.1 Inclusion Criteria

3.1.1 Adult men diagnosed with prostate cancer and undergoing prostatectomy as treatment of choice
3.1.2 Index lesion visible on multiparametric MRI confirmed by targeted biopsies using KOELIS Trinity®
3.1.3 Patient suitable for IV sedation or general anesthesia and TMA
3.1.4 Free, informed, and written consent, dated and signed before the enrollment and before any exam required by the trial

3.2 Exclusion Criteria

3.2.1 Past medical history of prostate surgery
3.2.2 Past medical history of radiotherapy or pelvic trauma
3.2.3 Past treatment for PCa (radiation, ablation, ADT, chemotherapy)

4.0 TREATMENT PLAN

4.1 Treatment Dosage and Administration

Microwave: Microwave radiation refers to the region of the electromagnetic spectrum with frequencies from 900 to 2450 MHz. Microwave can also be referred as “Ultra high radiofrequency” given the possible wavelength spectrum overlap. Water molecules have asymmetric electric charges. The alignment and the charges on the atoms are such that the hydrogen side has a positive charge, and the oxygen side has a negative charge. For a microwave oscillating at 9.2×10^8 Hz, the charge changes signs nearly 2 billion times per second. When an oscillating electric charge interacts with a water molecule, it causes the molecule to flip. Microwave is specially tuned to the frequency of water molecules to maximize this interaction. As a result of the microwave radiation hitting the water molecules, the water molecule flips back and forth 2–5 billion times per second depending on the frequency of the microwave energy. The vigorous movement of water molecules raises the temperature which is a measure of how fast molecules move. Therefore, electromagnetic microwaves heat matter by agitating water molecules in the surrounding tissue, producing friction, raise temperature, and induce cellular death via coagulation necrosis]. This is one major difference between microwave that treat simultaneously a whole volume and other energies relying on radial conduction of the energy from the antenna (cryotherapy, radiofrequency).

We use MedWaves Avecure generator and associated microwave antenna, used for thermal microwave ablation. Under general anesthesia and ultrasound guidance, the prostate is visualized, and the optimal approach is determined. A thin (16-gauge) microwave antenna is then placed directly into the targeted area. When the antenna is attached to the microwave generator with a coaxial cable, an electromagnetic microwave is emitted from the noninsulated portion of the antenna. The

generator produces 10-60W of power at a frequency of 902-928 MHz. Intratissue temperatures can be measured with built-in temperature sensor [33].

MRI/US fusion and OBT-Fusion®: Targeted Microwave Ablation (TMA) is the specific wording when microwave is combined with an MRI/US fusion guidance system, the latter providing improved planning, guiding and visualization capabilities compared with conventional ultrasound systems. During the trial the KOELIS Trinity® system will be used. This 510k-cleared medical device embeds four key technologies that are relevant for focal therapy using microwave:

- 3D Ultrasound using motorized ultrasound probes that allow to reconstruct the prostate in 3D in a few seconds with no probe movement. The ultrasound probe comes with guiding accessories (needle guides) to offer improved guidance of the diagnostic or therapeutic instrument in the prostate
- Elastic MRI/US fusion allowing to precisely fuse a preoperative MRI containing relevant tumor information (location, size, aggressiveness) with intraoperative 3D Ultrasound to allow the physician to guide the needles directly inside the index tumor. This technology is necessary given that direct in-bore MRI guidance is cost and time consuming.
- OBT-Fusion® is an image-based tracking technology that tracks and helps navigate the needle inside the prostate during the whole intervention. A patented algorithm then recognizes the needle location in a reference 3D ultrasound volume acquired at the beginning of the exam. The advantages are an increased accuracy due to the automatic patient and prostate movement during the exam, an absence of need of further sensors that are usually required to have such navigation capability, and an automatic recalibration throughout the exam instead of manual recalibration
- 2nd Look is a specific KOELIS technology that allows to recall the biopsy mapping information during a second intervention, which can typically be a focal treatment. The recalled information include the number of targeted and systematic cores, the location of MRI lesions that were biopsied, the histological features of each individual cores. The key advantage is the ability to further personalize the treatment plan according to each patient, rather than relying only on the MRI information.

Dr. Andre Abreu who is an image-guided focal therapy expert will be delivering the TMA ablation for all the interventions[39]–[42].

4.2 Toxicities and Dosing Delays/Dose Modifications

We will monitor for TMA related toxicities such as rectal injury, bladder injury, and perineal pain. Complications will be managed with best supportive care. Since previous literatures have not adapted similar study design (TMA and immediately resect), we do not have standard dosage of TMA needed to ablate planned area on the prostate. Therefore, we start from low output and ablation time and escalate them in accordance with corresponding histologically ablated area.

According to the manufacturer's data based on other soft tissues, ablation coverages of short x long axis (cm) are estimated 1.5 x 2.5 for short-time (3min) ablation, 2.0 x 2.7 for medium-time (5min) ablation, and 2.5 x 3.0 for long-time ablation.

Based on the data, our TMA delivery strategy is planned as follows.

Immediate resection phase

1st patient
Template #1
Right lobe: Single Small (3min) Ablation
Left lobe: Single Small (3min) Ablation
2nd patient
Template #2
Right lobe: Single Medium (5min) Ablation
Left lobe: Single Medium (5min) Ablation
3rd patient
Template #3
Single lobe: Single Large (7.5min) Ablation
4th patient
Template #4
Single lobe: Spatially-overlapped Double Medium-time (5min) Ablation
5th patient
Template #5
Single lobe: Spatially-overlapped Double Large (7.5min) Ablation

Delayed resection phase

6-7th patient
Template #1
8-9th patient
Template #2
10-11th patient
Template #3
12-13th patient
Template #4
14-15th patient
Template #5

In order to avoid collateral damage and for safety, the TMA delivery protocol may be modified by the physician's discretion but still in accordance with the manufacturer's recommendation.

4.3 Concomitant Medications/Treatments

NA.

4.4 Other Modalities or Procedures

4.4.1 **Radical prostatectomy**

First 5 patients undergo RP immediately after TMA under the same anesthesia as their definitive treatment for PCa.
Following 10 patients undergo RP one month after TMA under the general anesthesia as their definitive treatment for PCa This surgery is performed as standard of care.

4.4.2 **Duration of Therapy**

One session (one entire TMA procedure consisting of one to two ablations in accordance with TMA delivery protocol) only.

4.5 Removal of Patients from Protocol Therapy

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation of treatment will be documented and may include:

- Patient withdraws consent
- Patient is unable to comply with protocol requirements
- Patient experiences toxicity that makes continuation in the protocol unsafe
- Treating physician determines continuation on the study would not be in the patient's best interest

Notify the Principal Investigator and document the reason for study removal and the date the patient was removed from treatment in the Case Report Form. The patient should be followed-up with per protocol.

4.6 Duration of Follow Up

The study will begin at the time of informed consent and ends at 30 days (± 15 days) visit following RP. No further follow-up is required as part of this study.

4.7 Patient Replacement

Patients may be removed from the study at any time either via patient preference or failing to undergo either TMA or RP. If a patient is removed from the study, an additional patient may be enrolled. Patients who complete all procedures outlined in this protocol will be considered "fully evaluable", whereas those who fail to undergo any of the procedures (i.e. TMA or RP) will be considered "not fully evaluable". We will report the numbers and percent of men considered "not evaluable". If there are more than 1 or 2, we will try to figure out if there are any salient characteristics that distinguish "fully evaluable" from "not fully evaluable" patients.

5.0 STUDY PROCEDURES

5.1 Screening Phase and Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done during initial clinical visit. It is acceptable to allow assessments performed

per standard clinical indications to be used for baseline values, but these again should be done within the screening window of 90 days or need to be repeated.

The screening procedures include:

Routine procedures done for patient's medical care:

5.1.1 Medical history

Complete medical and surgical history.

5.1.2 Demographics

Age

5.1.3 Review subject eligibility criteria

5.1.4 Review previous and concomitant medications

5.1.5 Physical exam and standard perioperative blood tests

Vital signs (temperature, pulse, respirations, blood pressure), height, weight. Bloods include full blood count, renal function and coagulation profile, tumor markers.

5.2 Randomization

There will be no randomization in treatment allocation.

5.3 Treatment Procedures

5.3.1 Registered into study

Once informed consent is obtained, patients will be registered into the study. We describe below all procedures that correspond to the treatment phase of our study: "TMA and RP phase" for the first 5 patients and "TMA phase" and "RP phase" for the following 10 patients (refer to study schema).

First 5 patients

"TMA and RP PHASE"

This phase of the study will be initiated within 30 days of registration.

5.3.2 Pre-TMA MRI *(Only performed if not done within previous 6 months)*

- ***mp-MRI technique*** mp-MRI will be performed using a 3-T MR-750 MR-scanner (General Electric, Waukesha, WI, USA) with a pelvic 16-channel phased-array body coil. Apparent diffusion co-efficiency (ADC)-map in diffusion weighted images (DWI) with the same orientation as transverse T2 weighted (T2-w) images. Dynamic contrast enhanced (DCE) image data will be post-processed with pharmacokinetic analysis software (iCAD, Nashua, New Hampshire). Lesions suspicious for clinically significant cancer in T2w, ADC-map, iCAD-DCE will be scored on a Prostate Imaging Reporting and Data System version-2.1 (PIRADS v.2.1)

scale from 1-5 by dedicated radiologists with expertise in prostate MRI [43]. T2w images will be manually segmented, the prostate outlined in each MRI plane and converted into a 3D object using specialized software. The final 3D prostate model will be imported into a 3D-design software (SolidWorks Corp., Concord, MA, USA) where it will be subtracted from a pre-generated rectangular mold to create an internal cavity that mirrors the patient's prostate on MRI. The mold will be created by 3D printer (Dimension Elite 3D printer, Stratasys, Inc., Eden Prarie, MN, USA), and will be used for treatment planning.

5.3.3 TMA

TMA preparation: The patients are instructed to self-apply rectal 2 enemas: one the night before, one the morning of TMA; nothing per oral night prior (8hs) to TMA.

Patient position and probe position: The patient is brought into the operating room and general anesthesia is obtained. Prophylactic antibiotic (Levaquin 500mg IV) is administered. The patient is placed in lithotomy position. The genitalia and perineal are prepped and draped in a sterile fashion. Digital rectal exam is performed. The 3D Side Fire endocavity TRUS probe (K3DEL00, Koelis, Meylan, France) is then inserted into the rectum.

Ablation planning: The prostate is TRUS-scanned, the transverse and longitudinal images are acquired, the dimensions of the prostate are measured and the 3D volume of the prostate is acquired. The ultrasound (US) images are adjusted to provide optimal visualization of the prostate, rectal wall and surrounding tissues. The contours of the prostate are drawn to delineate the prostate contour, the urethra contour, the rectal wall contour. After fusion with the preoperative MRI, the ablation zones set by the manufacturer can be visualized in 3D as a simulation prior to the needle insertion.

TMA delivery and monitoring: Under real-time TRUS guidance, a microwave antenna is transperineally inserted into the planned-ablation area. As much as possible, the needle will be placed at the center of the index tumor to maximize the treatment effect inside the lesion. OBT-Fusion® guidance ensures the proper location of the needle with respect to the targeted index lesion. Once the needle location is confirmed and the optimal treatment parameters are chosen, the microwave generator is configured accordingly, and the treatment can start. The ablation typically lasts for a few minutes.

Post-treatment: After the procedure, the antenna and TRUS probe are withdrawn and the robotic RP as standard of care for the prostate cancer starts under same general anesthesia. Patients is sent to the recovery room for monitoring. After completely recovery from the anesthesia the patient stays hospital overnight or is discharged home with the urethral Foley in place. Prescriptions are provided for: bladder antispasmodic, anti-inflammatory, and antibiotics.

5.3.4 Pathologic Evaluation

Following prostatectomy, the prostates will primarily be evaluated using standard-of-care diagnostic methods, and secondarily be assessed in accordance with the thermal treatment parameters. The intact prostate specimens should be received in pathology within two up to six hours of prostatectomy. Standard gross examination of prostate specimens will be done including measurement, appropriate inking for margins and serial transverse sectioning from the bladder neck across the prostate to the penile urethral margin. Digitally photographing of these sections will be done. TTC viability staining will be performed not less than 2 hours after TMA and 4 up to 6 hours after completion of prostatectomy. Macroscopic evaluation of the TTC stained sections will be performed where viable tissues would exhibit maroon color change, while ablated non-viable tissues should show absence of color change. Routine tissue processing and H&E staining will be performed. H&E stained whole mount sections of the prostatectomy specimens will be evaluated on microscopy. If indicated, IHC staining will be done.

Following 10 patients

"TMA PHASE" and 1mo delayed "RP PHASE"

5.3.5 Pre-TMA MRI (Only performed if not done within previous 6 months)

5.3.6 *mp-MRI technique:* The same as the first 5 patients.

5.3.7 TMA

TMA preparation:
Patient position and probe position:
Ablation planning:
TMA delivery and monitoring: The same as the first 5 patients.
Post-treatment: After the procedure, the antenna and TRUS probe are withdrawn. Patients is sent to the recovery room for monitoring. After completely recovery from the anesthesia the patient stays hospital overnight or is discharged home with the urethral Foley in place. Prescriptions are provided for: bladder antispasmodic, anti-inflammatory, and antibiotics.

5.3.8 RP

30 days (±15days) after TMA, the robotic RP as standard of care for the prostate cancer starts under same general anesthesia. Just prior to RP, MRI/TRUS fusion transperineal biopsy will be performed under the same anesthesia of RP. Then the RP will be immediately carried out as per SOC. After RP, the patient is sent to the recovery room for monitoring. After completely recovery from the anesthesia the patient stays hospital overnight or is discharged home with the urethral Foley in place. Prescriptions are provided for: bladder antispasmodic, anti-inflammatory, and antibiotics.

5.4 Follow-up Procedures

First 5 patients

"Follow-up PHASE"

5.4.1 The patient returns to clinic in 10 (±5) days after TMA and immediate RP for removal of urethral Foley and formal voiding trial. An AE assessment is performed at this time.

"Completion PHASE"

5.4.2 Patients will do a follow-up visit 30 (±10) days after TMA and immediate RP per standard of care. Adverse effects will be assessed during this visit. Tests for perineal discomfort, PSA, IPSS, IIEF-5, EPIC-26, uroflowmetry and patient satisfaction questionnaire are assessed.

5.4.3 Adverse event assessment

Adverse events will be assessed. See section 7 for Adverse Event monitoring and reporting

Following 10 patients

"Follow-up PHASE 1"

After TMA, the patient returns to clinic in 10 (±5) days. An AE assessment is performed at this time.

"Follow-up PHASE 2"

5.4.4 After TMA, the patient returns to clinic in 30 (±10) days for IPSS, IIEF-5, EPIC26, uroflowmetry, PSA, MRI and AE evaluation. These will be performed prior to RP.

"Follow-up PHASE 3"

5.4.5 After RP, the patient returns to clinic in 10 (±5) days for removal of urethral Foley. An AE assessment is performed at this time.

"Completion PHASE"

5.4.6 Patients will do a follow-up visit 30 (±15) days after RP per standard of care. Adverse effects will be assessed during this visit. Tests for perineal discomfort, PSA IPSS, IIEF-5, EPIC-26, uroflowmetry, and patient satisfaction questionnaire are assessed.

5.4.7 Adverse event assessment

Baseline adverse events will be assessed. See section 7 for Adverse Event monitoring and reporting

5.5 Study Calendar

First 5 patients

	Screening visit	Treatment date			Follow-up Phase	Completion phase
		Day 0			Day 10 (±5)	Day 30 day (±10)
		OR *pre*-TMA	TMA	OR *post*-TMA		
Medical history	X					
Demographics	X					
Medication review	X					
Adverse Event assessment	X	X		X	X	X
Physical Exam	X				X	X
Vital Signs (temperature, pulse, respiration, BP, (*height and weight -only on screening*)	X				X	X
Hematology, full blood count, renal function and coags	X					
PSA measurement	X					X
Patient eligibility review, enrollment	X					
Informed Consent	X					
MRI	X					
Uroflowmetry	X					X
Catheter removal					X	
IPSS, IIEF-5 and EPIC-26 questionnaires	X					X
Urine analysis and culture	X					
Radical prostatectomy				X		
TMA delivery			X			
Pathology				X[1]		

1: Pathologic evaluation including TTC staining is performed immediately after RP.

Following 10 patients

	Screening visit	**Treatment date**			**Follow-up Phase1**	**Follow-up Phase2**	**RP date**	**Follow-up Phase3**	**Completion phase**
		Day 0			Day 10 (±5)	Day 30 (±5)[2]	Day 30 (±5)	Day 40 (±5)	60 days (±10 days)
		OR *pre*-TMA	TMA	OR *post*-TMA					
Medical history	X								
Demographics	X								
Medication review	X								
Adverse Event assessment	X	X		X	X	X		X	X
Physical Exam	X				X	X		X	X
Vital Signs (temperature, pulse, respiration, BP, (*height and weight -only on screening*)	X				X	X		X	X
Hematology, full blood count, renal function and coags	X								
PSA measurement	X					X			X
Patient eligibility review, enrollment	X								
Informed Consent	X								
MRI	X					X			
Uroflowmetry	X					X			X
Catheter removal					X			X	
IPSS, IIEF-5 and EPIC-26 questionnaires	X					X			X
Urine analysis and culture	X								
Intra-operative prostate biopsy							X		
Radical prostatectomy							X		
TMA delivery			X						
Pathology							X[3]		

2: The date of Follow-up Phase2 is scheduled 1-5 days before RP date.
3: Pathologic evaluation without TTC staining is performed after RP.

6.0 MEASUREMENT OF EFFECT

The main effect we will measure to achieve our primary objective is the treatment effect (proportion of dead tissue - benign and cancerous) inside the TMA-treated zone on the prostatectomy specimen. The excised prostate will be sliced at 4 mm intervals. Each slice will be embedded, and a pathologist will examine slides corresponding to each slice. The pathologist will be blinded to MR and TRUS imaging results. TTC and/or H&E staining are performed to determine thermal ablation zone size/location/relationship to urethra seminal vesicles capsule and other structures.

7.0 ADVERSE EVENTS

7.1 Experimental Therapy

7.1.1 One-month delayed resection

Prostate cancer is usually a slow-growing cancer even in the setting of high-risk disease. Therefore, delaying RP doesn't impact on earlier, medium- or long-term oncologic and functional outcomes [44-46]. On a period of one month from TMA to RALP, the functional outcomes should not be affected by the TMA. In fact, the one-month delay from TMA to RP is long enough to allow for decreasing peri-prostatic edema, however short enough to avoid tissue fibrosis, retraction and adhesion that would happen with prolonged delay from TMA. The functional outcomes should be dictated/related to RALP, patients' and cancer characteristics such as nerve sparing RALP technic, urethral length, patients age, etc. The TMA will not be performed with a curative intent, in fact, it will be performed conservatively, sparing the boundaries of the prostate to avoid damage to the neurovascular bundles and the rectum. Furthermore, there will be a dose-escalation approach, where the TMA will be performed from small to larger ablation areas allowing for precise evaluation of the ablated area in each planned ablation template.

7.1.2 Two-separated procedures

The risks of the operations are according to each operation individually. Focal therapy for prostate cancer (HIFU and Cryoablation) is routinely performed by USC urologists. In fact, we have one of the largest experiences with ablation therapy for PCa in the US [42,47]. These procedures are safe with low risk of perioperative complications and lower risks of severe adverse events. In our initial series of 100 consecutive men who underwent HIFU focal therapy for PCa, approximately 13% of the patient experienced adverse events being the most common urinary retention, requiring prolonged catheterization, and urinary infection, requiring antibiotics [39]. No patient underwent additional intervention due to complications/adverse events. Additionally, there were no anesthesia-related complications. It is important to note that these patients were treated with a curative intent with large areas of ablation often extending towards to the urethra. Recent trials investigating TMA also demonstrated the feasibility and safety [26-28]. For the current PRAMA study, the TMA approach will be more conservative because the intent isn't curative, therefore the urethra and surrounding organs will be preserved. As such, we expect lower chances of side effects. The anesthesia risks related to RALP is similar to the approximately 600

RALPs annually performed by USC urology. The anesthesia risk shouldn't be affected by the TMA prior to RALP.

7.2 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of Subjects enrolled in the studies. Adverse events are reported in a routine manner at scheduled times during a trial. For this study, the AEs will be monitored and recorded at the day of surgery and each postop visit, per study calendar. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

7.3 Definition

7.3.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. Based on pilot data, there are no adverse events associated with performing TMA in patients who are candidates for RP. The AEs of interest for this study include:
Acute urinary retention: any episode of urinary retention following surgery, needed catheterization.
Hematuria: Any gross blood/clot in the urine, needed bladder irrigation.

7.3.2 Severity of Adverse Events

All adverse events will be graded according to the Clavien Dindo Classification (CDC) in the post-operative prostatectomy setting (outlined below).
The Clavien Dindo classification is available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360123/
Adverse events are monitored and recorded in accordance with Common Terminology Criteria for Adverse Events (CTCAE) v5.0 as well, although the safety of the protocol is evaluated based on CDC.CTCAE v5.0 is available at
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50

Clavien Dindo Classification (CDC)

Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics and electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I

	complications. Blood transfusionsand total parenteral nutritionare also included.
Grade III	Requiring surgical, endoscopic or radiological intervention
IIIa	Intervention not under general anesthesia
IIIb	Intervention under general anesthesia
Grade IV	Life-threatening complication (including CNS complications)* requiring IC/ICU-management
IVa	Single organ dysfunction (including dialysis)
IVb	Multi organ dysfunction
Grade V	Death of a patient

7.3.3 Serious Adverse Events

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that:

1. Results in death
2. Life-threatening event
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions

7.3.4 Is life-threatening

Only SAEs that are judged by the Principal Investigator to be related or possibly related to TMA and not part of the expected post-operative course will be assessed and reported.

- The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

- Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly/birth defect
- Is an important medical event
- Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event".

For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.4 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the Clavien Dindo Classification (CDC).

Step 2: Grade the adverse event using the CDC.

Step 3: Determine whether the adverse event is related to the protocol therapy Attribution categories are as follows:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely—The AE is *unlikely related* to the study treatment.
- Unrelated – The AE is *clearly NOT related* to the study treatment.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.
Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in the current known adverse events including rectal injury, urethral injury, acute urinary retention, perineal pain and uncontrollable hematuria.

7.5 Reporting Requirements for Adverse Events

- **Expedited Reporting**
  - The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, that were possible related to the research procedures..

- The Institutional IRB will be notified in accordance with the institutional policy about any unanticipated problems involving risk to subjects or others (UPR).

  The following events meet the definition of UPR:

  1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
  2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
  3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
  4. Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
  5. Any breach in confidentiality that may involve risk to the subject or others.
  6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

- The USC NCCC Data and Safety Monitoring Committee (DSMC) must be notified within 24 hours of submission of such reportable event to the IRB. The patient ID and the study number as well as identifier of the SAE report should be submitted to the DSMC Coordinator via email or Fax to the attention of the DSMC Coordinator at 323-865-0089.

▪ **Routine Reporting**

- All other adverse events- such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission. For studies requiring USC DSMC review, this report should also be forwarded to the DSMC Coordinator. If USC holds the IND, a list of all toxicities will be included in the IND annual report.

7.6 Unblinding Procedures For Blinded and Randomized trials

- NA

7.7 Monitoring Rules for Safety

All other adverse events- such as those that are expected or are unlikely or definitely not related to the study participation- are to be reported as part of regular data submission.

We will pause study enrollment after 10 patients if 2 or more patients do not progress to RP and perform a thorough assessment as to ongoing safety of the study.

If a severe adverse event related to TMA occurs during TMA procedure or by follow-up phase (Day 10 ± 5) for immediate resection phase or by follow-up phase 3 (Day 40 ± 5) for delayed resection phase, we assess, discuss, and tabulate the severe adverse event.

To ensure safety, the next patient's TMA will be scheduled at least after follow-up phase of the previous patient for immediate resection phase. For delayed resection phase, the next patients for the different TMA template will be scheduled at least after follow-up phase 3 of the previous patients.

In addition, for the delayed resection phase, in the case that two in two patient who underwent the same TMA template experienced severe adverse events related to TMA, we stop to proceed the next TMA template.

We set 30% as clinically acceptable severe adverse events (defined as CDC ≥3) rate (i.e. less than 4 in 10 delayed resection cases).

8.0 DEVICE INFORMATION

All the investigational devices required in the PRAMA protocol to perform the TMA procedure are 510(k)-cleared and used in accordance with their respective indications for use, as described below:

- Based on the 510(k) of AveCure™ Ablation System (reference: K143203):
  "*The MedWaves AveCure™ Ablation System is intended for use in percutaneous, laparoscopic, and intraoperative coagulation-ablation of soft tissue. The MedWaves AveCure™ Ablation System is not intended for use in cardiac procedures.*"
- Based on the 510(k) of TRINITY / 3D PROSTATE SUITE (reference: K170521):
  "*TRINITY and its embedded 3D PROSTATE SUITE software are intended to be used by clinicians and their assistants, qualified to perform ultrasound diagnosis and ultrasound-guided procedures, in public of private hospitals. TRINITY is indicated to:*
  - *Generate ultrasound images for structural analysis and fluid flow analysis for*
    - *Urology,*
    - *Gynecology,*
    - *Vascular,*
    - *Abdominal,*
    - *Small organs,*
    - *Soft tissues and*
    - *Musculoskeletal exams*

TRINITY is not indicated for ophthalmic and cranial ultrasonography."

To summarize, the TMA procedure will be performed on soft tissue (prostate). The ablation will be achieved thanks to MedWaves AveCureTM Ablation System under ultrasound-guidance using TRINITY. Besides, the risks associated with this procedure are known, controlled and well-described, as previously outlined, qualifying the procedure as a non-significant risk procedure.

9.0 CORRELATIVES/SPECIAL STUDIES

9.1. Correlation of histology with MRI

Following prostatectomy, the prostates will primarily be evaluated using standard-of-care diagnostic methods, and secondarily be assessed in accordance with the thermal treatment parameters. The intact prostate specimens should be received in pathology within two up to six hours of prostatectomy. Standard gross examination of prostate specimens will be done including measurement, appropriate inking for margins and serial transverse sectioning from the bladder neck across the prostate to the penile urethral margin. Digitally photographing of these sections will be done. TTC viability staining will be performed not less than 2 hours after TMA and 4 up to 6 hours after completion of prostatectomy. Macroscopic evaluation of the TTC stained sections will be performed where viable tissues would exhibit maroon color change, while ablated non-viable tissues should show absence of color change. Routine tissue processing and H&E staining will be performed. H&E stained whole mount sections of the prostatectomy specimens will be evaluated on microscopy. If indicated, IHC staining will be done.

10.0 STATISTICAL CONSIDERATIONS

All patients who sign the consent and are registered will be accounted for. However only those patients who undergo TMA and who undergo the radical prostatectomy will be included in the primary analyses; these will be the fully evaluable patients. Those who fail to complete the protocol as planned will be summarized; reasons for failure to complete will be examined.

10.1 Summary of Design

This is a feasibility study to evaluate whether TMA is promising as a stand-alone local treatment for men with localized prostate cancer, thereby eliminating the need for prostatectomy or standard radiation as the primary treatment. For practical considerations, the total number of study participants is limited to 15 patients by 1) cost and 2) feasibility (could not do many more during the funding

period). The number of patients included may be extended considering the need to evaluate different treatment parameters (still according to manufacturer data).

10.2 Justification of Trial Design.

- The decision to evaluate at 5 patients in immediate resection phase would provide intraoperative safety and feasibility, and evaluation of the ablated area on the radical prostatectomy specimen assessed by TCC stanning according to TMA delivery protocols. The decision to evaluate at 10 patients in delayed resection phase would provide intraoperative safety and feasibility, in addition to estimate of the short-term safety after TMA, evaluation for functional status (urinary symptoms, potency, incontinence), PSA response, MRI evaluation, prostate biopsy evaluation, safety and feasibility of salvage prostatectomy after TMA, and evaluate tissue viability on HE stanning. Additionally, The post-TMA MRI and prostate biopsy performed just prior to the delayed radical prostatectomy will be used to predict/evaluate the actual prostatectomy findings.

10.3 Primary Objective Statistical Considerations

To determine the feasibility of transperineal targeted microwave ablation of an MRI-identified index prostate tumor in patients undergoing radical prostatectomy, we will evaluate the viable tissue remaining after TMA using histologic examination of excised prostate specimen.

10.4 Secondary Objective Statistical Considerations

The intra-operative safety of the targeted microwave ablation, the number of microwave ablations needed to ablate one mpMRI-visible lesion, the ease of use of the operative procedure and the feasibility of the immediate or 1 mo delayed radical prostatectomy after the targeted microwave ablation will also be evaluated.

10.5 Descriptive Objective Statistical Considerations

NA

10.6 Sample Size and Patient Accrual

Due to the nature of exploratory and feasibility study design, the sample size of 15 subjects was determined by practical considerations, including cost and feasibility (could not do many more during the funding period).

Through the immediate resection phase, 5 practical TMA templates and their ablation areas are evaluated. With following the delayed resection phase, the short-term safety and feasibility of TMA templates are evaluated.

The number of patients included may be extended considering the need to evaluate different treatment parameters (still according to manufacturer data).

10.7 Data Analyses Plans

All patients who sign the consent form will be registered and entered the database. For each patient, we will list whether he received the assigned protocol (and if not, the reasons), the outcome of the procedures, compliance with the protocol (and reasons for failure to comply), adverse events with any of the procedures, MRI results, and targeted biopsy results.

- The data from the immediate resection phase and delayed resection phase will be separately evaluated. The observed ablated area in immediate resected prostatectomy specimen based on TTC staining will be compared with the predicted ablation area according to the template. The tissue viability will be evaluated by HE staining on delayed resection prostatectomy specimen.
- The comparison of the difference between the treatment effect dimensions measured histologically on the prostatectomy specimen resected after TMA and the predictive ablation charts provided by the manufacturer will be performed. For this analysis, the data from immediate resection phase and delayed resection phase are separately evaluated.
- The comparison between the treatment effect dimensions measured histologically on the prostatectomy specimen resected one month after TMA, compared to post TMA dimensions measured on the pre-RP mpMRI.
- To evaluate the intra/post-operative safety of TMA, we defined 30% as clinically acceptable severe adverse events (defined as CDC ≥3) rate. If the severe adverse events occur less than 30% (equivalent to less than 4 cases in 10 cases) for the delayed ablation phase, the feasibility of TMA is proved.

Immediate resection phase

If a severe adverse event related to TMA occurs during TMA procedure or by follow-up phase (Day 10 ± 5), we assess, discuss, and tabulate the severe adverse event. For this evaluation, TMA-related adverse events include rectal injury, and urethral injury.

Delayed resection phase

If a severe adverse event related to TMA occurs during TMA procedure or by follow-up phase 3 (Day 40 ± 5), we assess, discuss, and tabulate the severe adverse event. In the case that two in two patient who underwent the same TMA template experienced severe adverse events related to TMA, we stop to proceed the next TMA template. For this evaluation, TMA-related adverse events include rectal injury, urethral injury, acute urinary retention, and uncontrollable hematuria.

Adverse events are also recorded in accordance with Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

- TMA procedure duration: probe-in / probe-out time, ablation time will be recorded.
- The ablation location in the prostate as seen in the KOELIS 3D map and the ablation location observed histologically on the prostatectomy specimen will be recorded.

- The change in functional outcomes between baseline and each follow-up visit, using IPSS, IIEF-5, EPIC-26 and uroflowmetry is recorded. For delayed resection phase, functional outcomes between baseline and follow-up phase 2 will be estimated as average change.
- The post-TMA MRI and prostate biopsy performed just prior to the delayed radical prostatectomy will be reported.

10.8 Reporting and Exclusions

- Evaluation of toxicity.

  We will monitor for TMA related toxicities such as rectal injury, urethral injury, acute urinary retention, perineal pain and uncontrollable hematuria. Complications will be managed as per standard of care in accordance with CDC.

- Evaluation of response. NA.

11.0 STUDY MANAGEMENT

11.1 Conflict of Interest

All investigators will follow the University conflict of interest policy. Any USC investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must complete a "Statement of Outside Interests Related to Research" Form. The application is reviewed and approved by the Conflict of Interest Review Committee (CIRC) USC conflict of interest policy is available at http://ooc.usc.edu/conflict-interest-research

11.2 Institutional Review Board (IRB) Approval and Consent Process

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol and all study related documents used in the study (e.g. QOL questionnaire, pill diary, brochure, advertisement etc).

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing a dated IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person authorized to obtain the informed consent

11.3 Required Documentation (for multi-site studies)

Not required

11.4 Registration Procedures

USC Registration:

The Research Coordinator must complete the protocol eligibility form to ensure that the patient is eligible. The PI will review the patient eligibility (with assistance from the Research Coordinator- who will assemble the required source documents, and do an initial review) prior to registering the patient on study.

The Research Coordinator or data manager will then register the patient into the Cancer Center database, Café, by accessing the Registration forms. Likewise, after the patient has completed the study, the Off Study forms in cafe will need to be completed, for Off Treatment and Off Study.

11.6 RECORDS AND DATA SUBMISSION

A. Confidentiality of Records

The original data collection forms will be kept in secure file cabinets, for USC patients forms will be kept in the Clinical Investigations Support Office (CISO).

B. Patient Consent Form

At the time of registration, signed and dated copies of the patient Informed Consent with the Human Rights and the HIPAA authorization must be given to the patient. Institutional policy regarding distribution and location of original consent documents should be followed. When a study is opened at two or more institutions, a copy of the signed consent and HIPAA should be sent to USC CISO QA team as soon as possible, and not later than within 5 business days of obtaining consent. For patients consented at USC/LAC, institutional policy should be followed: a copy of ICF and HIPAA should be uploaded through True to USC CRO and to CISO QA Team. The original will be kept in the patient research chart maintained by the study assigned Data Manager.

C. Registration Eligibility Worksheet

At the time of registration, the completed Eligibility Worksheet will be submitted to the QA Monitor at CISO for review of eligibility compliance.

D. Data Collection Forms and Submission Schedule

If a treatment trial, protocol data will be entered into eCRFs in Café.
Within two weeks of registration, the data manager will complete the initial set of On Study forms and baseline Toxicities
Within two weeks of completion of each course of treatment, the data manager must complete the Course Assessment, Toxicities, and if appropriate Response data.

- After Off Treatment, within two weeks of each follow up, complete the Follow Up forms.

11.7 Data Management and Monitoring/Auditing

11.7.1 Active Monitoring Program Details

a. **Adherence to Protocol/Per Patient**: It is the responsibility of the Principal Investigator (PI) to ensure that patient recruitment and enrollment, treatment, follow-up for toxicities and response, and documentation and reporting are all performed as specified in the protocol.

b. **Day-to-Day Monitoring – Eligibility:** The Study Coordinator will assist the investigator in reviewing eligibility and will assemble the required source documents, and do a final review by completing an Eligibility Registration Worksheet.The Eligibility Registration Worksheet with a copy of Informed Consent and supporting source documents will be submitted to CISO QA via email or Fax for verification prior to registering the patient on study.

c. **Day-to-Day Monitoring – Informed Consent:** Prior to registering the patient on study, the Study Coordinator will review the informed consent, to ensure that the patient has signed and dated the most current IRB-approved form, and that the form has been signed and dated by the person obtaining the consent as well as appropriate witnesses. A copy of the ICF will also be provided to CISO QA for review. CISO SOP 3.3 will be followed.

d. **Day-to-Day Monitoring – Treatment:** The PI and co-investigators are responsible for ensuring that treatment is given per protocol. The Study Coordinator will review the treatment orders with the treating investigator. The treating investigator will review the status of each patient on-study, with the Study Coordinator and the treating physician, on an on-going basis.

e. **Data Management – Patient Charts:** At USC, all written source documents not associated with the study research are maintained in the patient chart, which is stored in the Department of Medical Records at the appropriate hospital. At the Norris Hospital, the official medical record is the Electronic Patient File (EPF). Radiographical images are stored in the Department of Radiology and in an electronic system called Synapse. These are the permanent, official documents for each patient on-study. A copy of the signed informed consent, physician's notes, orders, test results and pathology notes are maintained in the patients' hospital charts. It is the responsibility of the research staff to ensure that the patient chart contains the required documents and work closely with treating investigators to ensure all protocol-related assessments are carefully documented.

f. **Data Management – Research Charts:** To facilitate adherence to the protocol schedule and data management, research charts are created to collect copies of the relevant notes, orders and results, that are in the Patient Chart. In Addition, all source documents related to the research, such as original informed consent forms, HIPAA Forms, AE assessment worksheets, disease response worksheets and NTFs are maintained in the Research Charts. Protocol calendars, worksheets, and checklists, are also kept in the research chart. These are maintained in the Clinical Investigation Support Office until the study is completed and the results are published and no further need is anticipated.

These are then stored off-site. It is the responsibility of the Data Manager to ensure that the research chart contains all the required documents.

g. **Data Management – Case Report Forms:** It is the responsibility of the Data Manager to complete the required case report forms. The case report forms are developed for the trial and these are used to finalize the data entry screens in the Cancer Center clinical trials database. It is the responsibility of the PI to review the Off-Study Summary form which summarizes pertinent toxicity, response and adherence information, once the patient has completed treatment.

11.7.2 Quality Assurance Monitoring Committee (QAMC) Oversight

The Quality Assurance and Monitoring Committee (QAMC) of the NCCC has the responsibility for study auditing and monitoring for protocol compliance, data accuracy, performance of audits and monitoring of accrual. QAMC procedures are detailed in the NCCC Data Safety and Monitoring Plan available on CISO Website.

11.7.2.1 QAMC Annual Patient Audits

The QAMC is responsible for conducting audits and providing the initial review of the audits. The trial is audited by the QAMC once a year. Faculty and staff at the Cancer Center involved in clinical research – but not directly involved in the research under evaluation – are asked to serve as auditors. Twenty percent of patients accrued during the past 12 months – and a minimum of 2 patients – are selected at random; however, additional patients may be selected for audit if there is some indication that there might have been a problem or unusual circumstance (possibly related to compliance, toxicity, response or some indication of an irregularity). The audit involves a review of the research chart, hospital medical record (i.e., source documentation) and evaluates the following: documentation of eligibility (including failure to obtain appropriate informed consent) and baseline status of the patient; documentation of adherence to protocol-specified treatment and follow-up; evaluation of toxicity; and evaluation of response or other outcome. In addition, for Institutional, Investigator Initiated Trials, Data in the CAFÉ database are compared to the information in the medical record.

11.7.2.2 QAMC Annual Protocol Review

All open trials are reviewed at least once a year by the QAMC (or more often if stipulated by the CIC). This annual review includes the following: evaluation of the current accrual relative to the planned total accrual; examination of gender and minority accrual; examination of all reported violations; review of past audits and correspondence with the PI; review of results of current audit (by an outside agency or by the NCCC QAMC); review of previous correspondence between the PI and the QAMC/DSMC. The QAMC review process is detailed in USC NCCC DSM Plan available on the CISO website.

11.7.3 Data and Safety Monitoring Committee (DSMC) Oversight

The Data and Safety Monitoring Committee (DSMC) is an independent body responsible for the safety of study subjects through the review of new protocols to ensure an adequate adverse event assessment/reporting plan, study stopping rules and through the real-time and periodic monitoring of severe adverse events (SAEs) or those AEs that

require expedited reporting. The DSMC performs quarterly and annual safety reviews as well as interim efficacy/futility analyses on institutional trials. DSMC procedures are detailed in USC NCCC DSM Plan available on the CISO website.

11.8 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.8.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within five (5) business days of making the change.

11.8.2 Non-Emergency departures from protocol

A protocol deviation is any variance from an IRB approved protocol.
If the deviation meets all of the following criteria, it is considered a minor protocol deviation that:

- Is generally noted or recognized only after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

If the deviation meets any of the following criteria, it is considered a protocol violation:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious noncompliance with federal regulations, State laws, or University policies.

Protocol Deviations: personnel will report to any sponsor or data and safety monitoring committee in accordance with their policies.

Protocol Violations: All protocol violations will be entered in the clinical trial database by the Research Coordinator. In addition, Research Coordinator and Investigator should report all protocol violations within one (1) week of the knowledge of the event using iStar.

11.8.3 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB as well as to all the sponsoring agencies (FDA, NCI, etc.) for review and for approval prior to implementation. It is the responsibility of the study PI to ensure that the appropriate agencies have been informed of the proposed amendments and that these have been reviewed and approved.

11.9 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.10 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion. Moreover, the Principal Investigator will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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13.0 APPENDICES

Ablation Delivery Strategy