Phase IIa Dose Escalation Trial of NanoPac Focal Therapy for Prostate Cancer in Subjects Undergoing Radical Prostatectomy

Protocol Identifying Number: NANOPAC-2016-02

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IND Sponsor: NanOlogy, LLC.

Version Number: 3.0

Date: 08 August 2017

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ASP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CLIA	Clinical Laboratory Improvement Amendments
CO2	Carbon Dioxide
CRF	Case Report Form
CRO	Clinical Research Organization
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-Limiting Toxicity
DSMB	Data Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture System
FDA	The U.S. Food and Drug Administration
GCP	Good Clinical Practice
Hct	Hematocrit
Hgb	Hemoglobin
HIFU	High Intensity Focused Ultrasound
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IFU	Instructions for Use
IND	Investigational New Drug Application
IP	Intraperitoneal
I-PSS	International Prostate Symptom Score Questionnaire
IRB	Institutional Review Board
IRE	Irreversible Electroporation
IV	Intravenous
LDH	Lactate Dehydrogenase
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mpMRI	Multiparametric MRI
MRI	Magnetic Resonance Imaging
MR-TRUS	Magnetic Resonance Imaging-Transrectal Ultrasound Fusion
NCI	National Cancer Institute
NDA	New Drug Application (Marketing Application)
OHRP	Office for Human Research Protections

PCA	Precipitation with Compressed Antisolvents
pCR	Pathologic Complete Response
pH	Hydrogen Ion Concentration
PI	Principal Investigator
PK	Pharmacokinetics
PSA	Prostate-Specific Antigen
PT	Prothrombin Time
PTT	Activated Partial Thromboplastin Time
RBC	Red Blood Cell
SAE	Serious Adverse Event
SDLC	Systems Development Life Cycle
SHIM	Sexual Health Inventory for Men Questionnaire
SOC	Standard of Care
SOP	Standard Operating Procedure
TEAE	Treatment-emergent Adverse Event
TRUS	Transrectal Ultrasound
UP	Unanticipated Problem
USC	University of Southern California
UTI	Urinary Tract Infection
WBC	White Blood Cell
WHO	World Health Organization

SPONSOR SIGNATURE PAGE

Protocol Title:	Phase IIa Dose Escalation Trial of NanoPac Focal Therapy for Prostate Cancer in Subjects Undergoing Radical Prostatectomy
Protocol Number:	NANOPAC-2016-02
Version Number:	3.0
Date:	08 Aug 2017
IND Number:	132694
Study Agent:	NanoPac® (sterile nanoparticulate paclitaxel) Powder for Suspension
Sponsor:	NanOlogy, LLC. 231 Bonetti Dr., Suite 240 San Luis Obispo, CA 93401-7310 805-595-1300

The Sponsor for IND 132694, NanOlogy, LLC, has transferred responsibility for all obligations set forth in 21 CFR Part 312 to US Biotest, Inc., with regard to the IND; in accordance with 21 CFR Part 312, US Biotest, Inc., shall be considered the sponsor with regard to all activities related to the IND.

SIGNATURE

Sponsor's Representative - Name and Title:
Gere diZerega, MD
President & CEO, US Biotest, Inc.

Signature of Sponsor's Representative

15 August 2017
Date

STATEMENT OF COMPLIANCE

I have read the attached protocol number NANOPAC-2016-02 entitled, *Phase IIa Dose Escalation Trial of NanoPac Focal Therapy for Prostate Cancer in Subjects Undergoing Radical Prostatectomy*, Version 3.0 dated 08 August 2017 and agree to comply with the contents of this document.

I agree to comply with applicable FDA regulations and guidelines set forth in 21 CFR Parts 11, 50, 54, 56, and 312.

This document is a confidential communication of US Biotest, Inc. The recipient agrees that no unpublished information contained herein will be published or disclosed without prior written permission of US Biotest. However, this document may be disclosed to appropriate institutional review boards, ethics review committees, or authorized representatives of the Investigator or of boards of health under the condition that they are requested to respect the confidentiality of the document.

The signature of the Principal Investigator below constitutes his/her agreement.

_____ _____

Signature of Principal Investigator Date

_____

Printed Name of Principal Investigator

PROTOCOL SUMMARY

Title: Phase IIa dose escalation trial of NanoPac focal therapy for prostate cancer in subjects undergoing radical prostatectomy

Précis: In this open-label, dose rising, Phase IIa trial with an expanded cohort at the dose of NanoPac determined to have the best tolerability and safety profile, subjects with prostate cancer scheduled for prostatectomy will have NanoPac injected under image guidance directly into the lobe of the prostate with the dominant lesion four weeks prior to prostatectomy. The study will include a dose escalation phase and a dose confirmation phase.

In the dose escalation phase, NanoPac concentrations of 6, 10, and 15 mg/mL in an injection volume of 20% of the lobe of the prostate containing the dominant lesion will be studied in cohorts of three, with cohorts enrolled sequentially starting at the lowest concentration. Following Data Safety Monitoring Board (DSMB) review of the cohort data the next cohort may begin enrolling, an additional three at the current dose may be enrolled, or if the first dose does not provide adequate safety and tolerability the study may be halted. The dose determined to be the most suitable for further evaluation, defined as the highest dose with an acceptable safety and tolerability profile as determined by the DSMB, will enroll additional subjects to provide a cohort of 12 subjects at that dose level.

Tumor volume and serum prostate-specific antigen (PSA) will be determined prior to NanoPac injection. Pharmacokinetic samples, PSA, and ejaculate will be collected in the interval between injection and prostatectomy. Imaging with multiparametric MRI (mpMRI) will be performed within three months prior to consent and again prior to prostatectomy. Prostate and pelvic lymph nodes excised at prostatectomy will be evaluated.

Objectives: Primary objective:

- To evaluate the safety and tolerability of NanoPac injected directly into the lobe of the prostate containing the dominant lesion.

Secondary objectives:

- To describe the pharmacokinetics (PK) of NanoPac injected directly into the lobe of the prostate containing the dominant lesion;
- To determine the effect of NanoPac on the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes.

Endpoints: Primary endpoint:

- Safety and tolerability, as demonstrated by adverse events (AE), changes in laboratory assessments, physical examination findings, and vital signs.

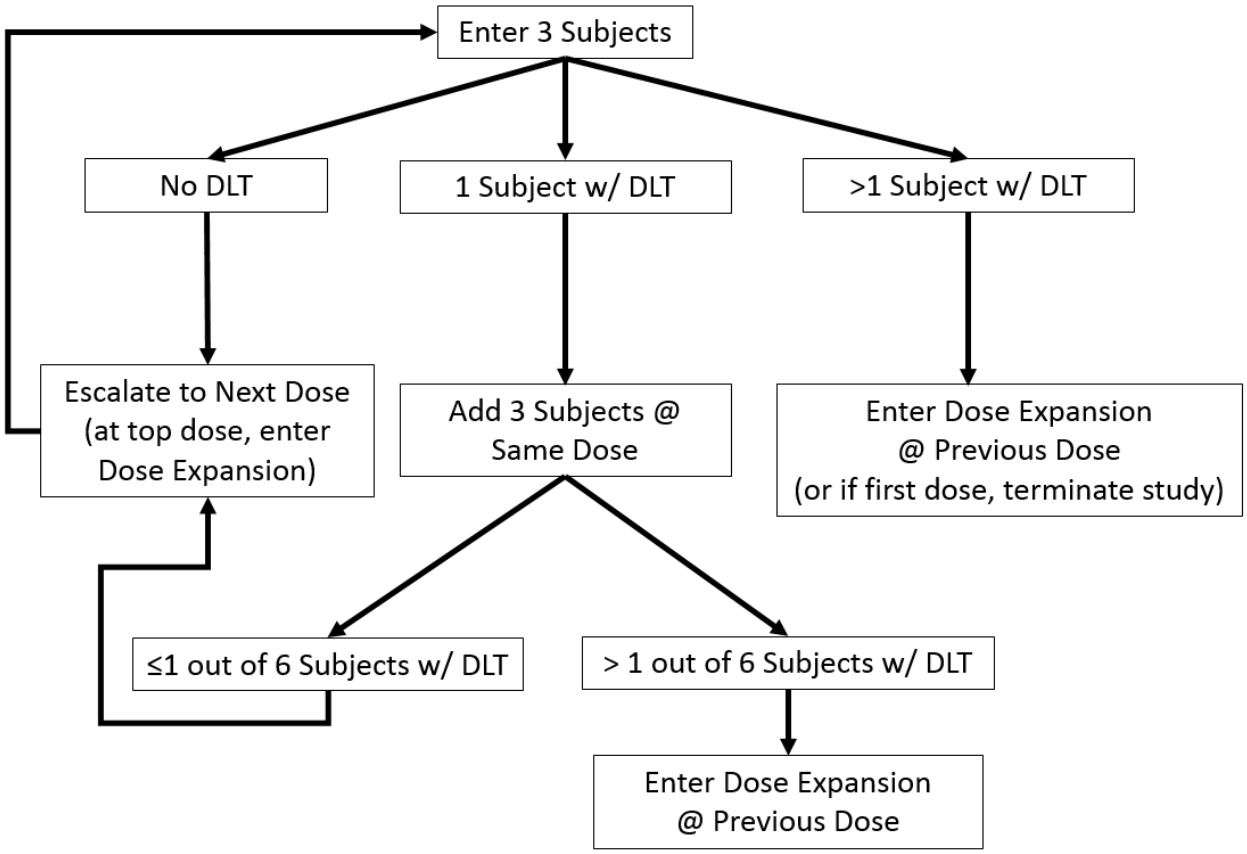
Secondary endpoints:

- Concentration of paclitaxel in the systemic circulation post-injection and prior to prostatectomy;
- Presence of paclitaxel in the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy;
- Tumor response (change in image volume on mpMRI; histologic evaluation via biopsy);

- Presence of tumor cells in the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy.

Population:	Up to a maximum of 30 men with adenocarcinoma of the prostate scheduled for radical prostatectomy
Phase:	Phase IIa
Number of Sites Enrolling Participants:	Up to two
Description of Study Agent:	NanoPac® (sterile nanoparticulate paclitaxel) Powder for Suspension ("NanoPac") for direct injection into the lobe of the prostate containing the dominant lesion at concentrations of 6, 10, 15 mg/mL in an injection volume of 20% of the lobe of the prostate containing the dominant lesion.
Study Duration:	The study duration will be up to 12 months.
Participant Duration:	The study duration is estimated to be approximately 9 weeks for each subject. Additional follow-up may occur.

SCHEMATIC OF STUDY DESIGN



1 KEY ROLES

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Laboratory – Prostate Tissue Tumor Cells

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

The Sponsor for IND 132694, NanOlogy, LLC, has transferred responsibility for all obligations set forth in 21 CFR Part 312 to US Biotest, Inc., with regard to the IND. In accordance with 21 CFR Part 312, US Biotest, Inc., shall be considered the sponsor with regard to all activities related to the IND. Therefore, references to "Sponsor" hereafter in this protocol refer to US Biotest, Inc.

Name and description of study agent:
Under IND 073529, NanOlogy, LLC has produced a formulation of nanoparticulate paclitaxel, identified as NanoPac® (sterile nanoparticulate paclitaxel) Powder for Suspension ("NanoPac"), which is the subject of this protocol. US Biotest, Inc. ("US Biotest") is developing NanoPac for the investigational treatment of adenocarcinoma of the prostate via direct injection. NanoPac, previously called Nanotax®, is manufactured using a Precipitation with Compressed Antisolvent (PCA) technique that employs supercritical carbon dioxide and acetone to generate paclitaxel nanoparticles within a well-characterized particle-size distribution. Following PCA, NanoPac is filled into 60 mL Type 1, USP, clear-glass vials (306 mg/vial), each of which is closed with a bromobutyl rubber stopper and aluminum crimp seal, and sterilized by gamma irradiation. Prior to administration at the hospital/clinic, NanoPac will be reconstituted with 1% Polysorbate 80, *NF* in 0.9% Sodium Chloride for Injection, *USP*, to form a suspension. The suspension will be further diluted with 0.9% Sodium Chloride for Injection, *USP* to achieve the final clinical formulation. This reconstitution and dilution will occur at the clinical site's pharmacy.

Nonclinical Summary:
US Biotest has completed two nonclinical studies of NanoPac in the PC-3 human prostate carcinoma nude mouse tumor xenograft model, Study PC3-e317 and Study PC3-e319.

In Study PC3-e317, NanoPac 37.5 mg/kg, qwk x 1 dose; 12.5 mg/kg, qwk x 3 doses; and 37.5 mg/kg, qwk x 3 doses were compared to vehicle and paclitaxel 30 mg/kg, qwk x 3 doses. Treatment with either a single dose or three once-weekly doses of NanoPac resulted in identical survival extension and an increased number of study survivors/regressions compared to vehicle. Dose dependent response could not be determined as dosing with NanoPac at 12.5 or 37.5 mg/kg qwk x 3 produced the maximum survival extension attainable in the study.

In Study PC3-e319, the efficacy of NanoPac was evaluated as part of a NanoDoce (nanodocetaxel) trial in female NCI Ath/nu mice. NanoPac was administered intra-tumorally (itu) at a dose of 37.5mg/kg qwk x 3 doses, and compared to vehicle (itu), NanoDoce (itu) 100mg/kg qwk x 1 dose, 37.5mg/kg qwk x 3 doses, and 100mg/kg qwk x 3 doses, and docetaxel 30mg/kg IV qwk x 3 doses. NanoPac reduced mean tumor volume by 74.1% by Day 22 compared to vehicle. Body weights for all groups remained stable or increased throughout the treatment phase.

Results for all NanoPac-treated animals were indicative of potential therapeutic activity. Additional data is presented in the NanoPac Investigator's Brochure.

Clinical Summary:

NanoPac has not been administered to humans via direct injection into the prostate. The only clinical study of NanoPac to date was Protocol HSC#11140, "A Phase I Study of Intraperitoneal Nanoparticle Paclitaxel in Patients with Peritoneal Malignancies." The results of this study were published by Williamson et al. (2015) in the journal *Cancer Chemotherapy and Pharmacology*.

Protocol HSC#11140 was a dose-escalating study evaluating intraperitoneally (IP) administered Nanotax (the same drug as NanoPac, but under a different name) at doses of 50-275 mg/m^2 given every 28 days until disease progression or unacceptable toxicity occurred. Twenty-two patients were enrolled in Protocol HSC#11140. IP administration of NanoPac did not lead to increases in systemic toxicity over that typically associated with IV paclitaxel. No Grade 2 or higher neutropenia and/or Grade 3 or higher neurologic toxicities were reported. Grade 3 thrombocytopenia, considered unlikely to be related to study agent, occurred in one patient. The peritoneal concentration-time profile of paclitaxel rose during the two days after dosing to peritoneal fluid concentrations 450-2900 times greater than peak plasma drug concentrations and remained elevated through the entire dose cycle. Best response assessments were made in 16 of the 21 subjects. Four subjects were assessed as stable or had no response and twelve patients had progressive disease. Five of 21 patients with advanced cancers survived longer than 400 days after initiation of IP NanoPac treatment.

Additional data from this clinical trial is presented in the NanoPac Investigator's Brochure.

Relevant Literature:

To date, clinical studies of paclitaxel injected directly into the prostate have not been published. However, several authors have reported results from human trials involving intraprostatic injection of various other agents (such as viral vectors) for the treatment of local prostate cancer (DeWeese et al. 2001; Johannsen et al. 2007a; Johannsen et al. 2007b; Finkelstein et al. 2012; Trudel et al. 2003; Pisters et al. 2004; Gulley et al. 2013; Kramer et al. 2001; Sonpavde et al. 2011; Patel et al. 2009; Shalev et al. 2000; Fujita et al. 2006; Belldegrun et al. 2001; van der Linden et al. 2005; Freytag et al. 2002). Further, intravenous administration of paclitaxel, often in combination with other agents and/or radiotherapy, has demonstrated efficacy for the treatment of locally advanced and metastatic prostate cancer (Sanfilippo et al. 2008; Hudes et al. 1997; Shepard et al. 2009; Sewak et al. 2010; Hussain et al. 2010; Urakami et al. 2002; Berry et al. 2004; Hudes et al. 1995).

Importance of the study:

Prostate cancer is the second most common cancer in men, second only to non-melanoma skin cancer. Despite the high prevalence of the disease, it is a constantly-evolving area of medicine, presenting difficult decisions for patients and healthcare providers. At present, treatment for prostate cancer consists primarily of either of two options: active surveillance or radical whole-gland therapy. However, this dichotomy fails to reflect the heterogeneity of prostate cancer and the nuanced patient experience. Due, in part, to the widespread adoption of PSA as a screening tool, more men are being diagnosed with lower-risk, lower-grade cancer (Marshall & Taneja, 2015). Active surveillance may be an appropriate choice for some of these men, as radical whole-gland therapy risks life-altering consequences, such as impotence and incontinence. This is supported by the statistic that 49% of men undergoing radical prostatectomy are found to have only insignificant or indolent cancer (Marshall & Taneja, 2015). Nonetheless, prostate cancer has the fourth highest mortality rate of any cancer, and 73% of patients initially enrolled on active surveillance who ultimately undergo prostatectomy are found to have a significant cancer (Marshall & Taneja, 2015). As such, active surveillance not only risks disease progression, but can be psychologically distressing to patients.

Focal therapy has emerged as a middle ground for prostate cancer treatment. Focal therapy treats localized prostate cancer while minimizing treatment-induced damage to adjacent structures. The goal of focal therapy is to maintain disease control at acceptable levels while preserving erectile, urinary, and rectal function by avoiding damage to the neurovascular bundles, external sphincter, bladder neck, and rectum (Miano et al. 2015). Commonly used modalities for focal therapy include cryotherapy, high intensity focused ultrasound (HIFU), irreversible electroporation (IRE), photodynamic therapy, and interstitial laser ablation. Aided by advanced imaging techniques to locate and target index tumors, focal therapy may be appropriate as an alternative or adjuvant to radical whole-gland therapies such as prostatectomy in certain patients with prostate cancer (Mendez et al. 2015).

2.2 RATIONALE

This Phase IIa study will include patients with adenocarcinoma of the prostate scheduled to undergo a prostatectomy. The study design allows for a safety evaluation of direct injection of NanoPac into the lobe of the prostate containing the dominant lesion as focal therapy prior to prostatectomy. We hypothesize that direct injection of NanoPac into the prostate will result in limited, if any, systemic exposure to paclitaxel and should therefore result in only low-grade and transitory AE.

A growing body of evidence supports the use of focal therapy for certain patients with prostate cancer. Existing focal therapies have demonstrated inconsistent efficacy and some risk of urinary and erectile complications. Taxanes, such as docetaxel and paclitaxel, have demonstrated antitumor activity against prostate cancer *in vitro* and *in vivo* (Axiak-Bechtel et al. 2013, Shikanov et al. 2008; van Soest & de Wit 2015). Direct injection, as opposed to intravenous (IV) administration, of paclitaxel would allow for higher concentrations of drug to target local disease with reduced systemic toxicity. Intraprostatic NanoPac is expected to be more effective than IV paclitaxel due to prolonged intraprostatic residence and dissolution, resulting in continuous and greater paclitaxel concentrations in the tumor site.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

There are no known potential risks from intratumoral injection of NanoPac. Nonclinical testing indicates that intraprostatic injection may lead to enlargement of the prostate which may cause urinary outflow obstruction.

2.3.2 KNOWN POTENTIAL BENEFITS

Paclitaxel, the active pharmaceutical ingredient of NanoPac, is effective in the treatment of metastatic prostate cancer. In two nonclinical studies of NanoPac in the PC-3 human prostate carcinoma nude mouse xenograft model, results for all NanoPac-treated animals were indicative of potential therapeutic activity. Additional data is presented in the NanoPac Investigator's Brochure.

3 OBJECTIVES AND PURPOSE

The primary objective of this study is to evaluate the safety and tolerability of NanoPac injected directly into the lobe of the prostate containing the dominant lesion in patients diagnosed with prostate cancer. The secondary objectives are to describe the pharmacokinetics (PK) of NanoPac injected directly into the lobe of the prostate containing the dominant lesion; and to determine the effect of NanoPac on the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

The proposed study is a Phase IIa, open-label, dose-escalating trial with an expanded cohort at the dose of NanoPac determined most suitable for further evaluation, defined as the highest dose with an acceptable safety and tolerability profile as determined by the Data Safety Monitoring Board (DSMB). Subjects will have NanoPac injected under magnetic resonance imaging-transrectal ultrasound fusion (MR-TRUS) guidance directly into the lobe of the prostate containing the dominant lesion. Four weeks after injection of NanoPac, the patient will undergo radical prostatectomy.

Prior to study entry, subjects will undergo ultrasound-guided prostate biopsy to diagnose and stage prostate cancer. This biopsy will be used to identify the dominant lesion, which is defined as the lesion with the highest Gleason score. The ultrasound performed during this biopsy will also be used to calculate the volume of the entire prostate. The volume of the lobe of the prostate containing the dominant lesion will be determined by calculating 50% of the total prostate volume as determined by ultrasound at the time of pre-study biopsy.

The study will include a dose escalation phase and a dose confirmation phase.

- Dose escalation: NanoPac concentrations of 6, 10, and 15 mg/mL in an injection volume of 20% of the lobe of the prostate containing the dominant lesion will be studied. Cohorts will be enrolled sequentially starting at the lowest concentration. Each cohort will have a planned minimum of three subjects. Data from the first three subjects in a cohort will be reviewed and evaluated by the DSMB. The outcome will be to determine whether to a) escalate to the next dose; b) add three additional subjects to the current dose; or c) expand the previous dose by three subjects.
- Dose confirmation phase: Once the dose deemed appropriate for expansion and further evaluation has been determined, subjects will be enrolled to that dose to provide a total of 12 subjects dosed at that level. If two doses are similar, subjects will be enrolled to each of the doses; however, the total number of subjects treated will remain 12.

4.1.1 PRIMARY ENDPOINT

The primary endpoint will be safety and tolerability, as demonstrated by adverse events (AE), changes in laboratory assessments, physical examination findings, and vital signs.

4.1.2 SECONDARY ENDPOINTS

The secondary endpoints will be:

- Concentration of paclitaxel in the systemic circulation post-injection and prior to prostatectomy;
- Presence of paclitaxel in the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy;
- Tumor response (change in image volume on mpMRI; histologic evaluation via biopsy);
- Presence of tumor cells in the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy.

4.1.3 EXPLORATORY ENDPOINTS

Not applicable.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

Patients who meet the following criteria will be considered eligible for participation in the study:

- Male;
- At least 18 years of age;
- Histopathologically proven adenocarcinoma of the prostate (Gleason grade ≥ 7);
- Considered to be candidate for radical prostatectomy and appropriate for treatment with paclitaxel therapy;
- Laboratory requirements:
  - WBC >2500/mm^3
  - Neutrophil >1500/mm^3
  - Hemoglobin >10 mg/dL
  - Platelet >100,000/ mm^3
  - AST and ALT <2.5 x ULN
  - Total bilirubin <1.5 x ULN
  - Creatinine <2 mg/dL
  - Normal PT/INR and PTT;
- ECOG of 0 or 1 (Appendix A);
- Indication for radical prostatectomy;
- Patient elects for radical prostatectomy;
- If sexually active, willing to use double condoms from time of NanoPac injection until prostatectomy;
- Signed informed consent;
- Patient must be willing to receive an mpMRI.

5.2 PARTICIPANT EXCLUSION CRITERIA

If a subject meets any of the following criteria, he must be excluded from the study:

- Evidence of locally advanced or metastatic disease;
- Prostate size ≥ 50 cc;
- Prior prostatectomy;
- Anticipated use of concomitant chemotherapy (other than the protocol specified agents), immunotherapy, or systemic use of hormonal therapy (such as GnRH analogs, antiandrogens, androgen receptor inhibitors, and 5-α reductase inhibitors) prior to surgery;
- Treatment with a prior investigational medication within 30 days of first dose of study agent;
- Any previous local treatment of the prostate (i.e., radiation);
- Any other condition (e.g., psychiatric disorder) that, in the opinion of the Investigator, may interfere with the patient's ability to comply with the study requirements or visit schedule;

- Known sensitivity to any of the study agent components;
- History of prior malignancy that has not been in remission for >5 years, with the exception of basal cell or squamous cell carcinoma.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Sufficient subjects will be screened to allow for up to 30 subjects to be enrolled in the trial. It is not anticipated that any advertising will be required for recruiting to the study. Subjects will be recruited from the Investigators' clinics and screened for eligibility and will proceed to treatment in groups of three.

Accrual of subjects will occur over a period of at least 6 months.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

Participants are free to withdraw from participation in the study at any time upon request. Their reason for wanting to withdraw will be documented in the source notes and in the Electronic Data Capture system (EDC).

- A subject who is not suitable to be treated will be withdrawn prior to Day 1 (Baseline/Treatment), and therefore in all study documentation this subject would be considered a screenfail subject.
    - Reasons for failing the screening will be documented in the source notes and in the EDC.

There is no reason currently anticipated that would cause the Investigator to terminate a subject's participation in the study. Once the treatment has been given it is very important that any events occurring be captured and followed for the safety of the subject.

- Subjects may be non-compliant with the study protocol in a way that much of the data is not captured which would usually require withdrawal for non-compliance. However, every attempt will be made to ensure the subject does come for surgery, and any data points missed would be considered "missing data." A subject would not be withdrawn in this situation.
- Clinical AE, laboratory abnormalities, or other medical conditions/situations may occur which would usually require withdrawal from a study. In this instance it is very important that all of these events be captured, followed, and documented, and therefore a subject would not be withdrawn but would continue to completion.

Should the Investigator feel it to be in the best interest of the subject for them to be withdrawn from the study, the Investigator will immediately contact the Medical Monitor to discuss the reasons for withdrawal.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

The Sponsor should be notified immediately when a subject is removed or withdrawn from the study after treatment with study agent, as every attempt should be made to capture as much information following treatment as possible.

In the event a subject is withdrawn, they would undergo final study visit evaluations (End-of-Study evaluations) which include vital signs, AE collection and concomitant medication updates.

Subjects that refuse or fail to appear for clinic visits and fail to respond to or cooperate with reasonable and diligent attempts at contact should not be discontinued from the study unless they fail to present themselves for surgery. In the event a subject is unable to be contacted and subsequently does not appear for surgery they should be considered lost-to-follow-up. Reasonable and diligent attempts such as dates and content of phone calls, emails and registered mail should be recorded in the subject's record.

If a subject repeatedly misses study visits or remains non-compliant between the time of NanoPac injection and prostatectomy, and where the majority of data is not available, the option to replace that subject in the cohort exists; however, the data that is collected from the non-compliant subject may still be used in the evaluations in this study.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminate if there is sufficient reasonable cause. Written notification, documenting the reason for the study suspension or termination, will be provided by the suspending or terminating party to Sponsor. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reasons for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants;
    - Routine Medical Monitoring determining a requirement for an ad hoc meeting of the DSMB, and/or routine DSMB reviews, will allow for termination of study based on unacceptable risk, which will consider all safety evaluations and dose-limiting toxicities (DLT).
    - In the dose escalation phase, the study may be terminated if in the first dose cohort one third of the subjects experience the same DLT (as defined in Section 6.1.7).
    - In the expansion phase of the study, if one third of the subjects (i.e., 4 subjects) experience the same DLT, the study will be stopped.
- Insufficient compliance with protocol requirements;
- Data that are not sufficiently complete and/or evaluable;
- Determination of futility.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the Sponsor, IRB, and/or Food and Drug Administration (FDA).

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

NanoPac will be manufactured by CritiTech, Inc. and provided for use in this study. Study agent will not be shipped to the study site until all regulatory documentation has been provided by the site, at which time the study agent will be released for shipment. Shipment will be via courier, temperature controlled 59˚ to 86˚F (15˚ to 30˚C), and will occur prior to site initiation. Study agent will be shipped to the on-site pharmacy where it will be stored according to the conditions required (see Section 6.1.3)

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

NanoPac is presented as a white powder, provided in a sealed vial within a study kit.

Study agent for all treatment groups will be supplied to the site in kits with one vial of Sterile Reconstitution Solution (1% Polysorbate 80, *NF* in 0.9% Sodium Chloride for Injection, *USP*) and one NanoPac 306mg powder-filled vial and one pre-printed Instructions For Use (IFU) insert in a 2ct kit. The site will be responsible for providing 0.9% Sodium Chloride for Injection, *USP* and lactated Ringer's solution.

Kits will be provided for a once-only use and will be assigned to one subject only. Reconstitution will occur at the pharmacy on-site and the reconstituted study agent will be delivered for use by the Investigator. An Instruction For Use (IFU) insert will be provided in each kit and an instructional video will be provided to each site prior to the initiation visit, ahead of the first patient being enrolled. The IFU will contain information on the reconstitution of the drug for all three dose levels, the storage of the drug once reconstituted, and the timeline permitted between reconstitution and use.

The vial will be labelled to include details as follows:

"NanoPac (sterile nanoparticulate paclitaxel) Powder for Suspension. 306mg per vial. Lot no.: XXXXXXXXXXX. Prior to and after reconstitution, store at 59˚ to 86˚F (15˚ to 30˚C). Caution: New Drug – Limited by federal law to investigational use. For single use only. Manufactured by: CritiTech Inc., 1849 East 1450 Road, Lawrence, KS, 66044."

The carton will be labeled with information indicating the content as follows:

"Each kit contains: 1 vial of NanoPac (sterile nanoparticulate paclitaxel) Powder for Suspension, 306 mg per vial; 1 vial Sterile Reconstitution Solution for NanoPac Powder for Suspension, 7 mL per vial; 1 instruction sheet for the reconstitution of the NanoPac dosing suspension and the dose withdrawal procedure."

6.1.3 PRODUCT STORAGE AND STABILITY

Prior to administration at the hospital/clinic, the dry, sterilized NanoPac vials will be stored at the pharmacy, temperature controlled at 59˚ to 86˚F (15˚ to 30˚C).

Once the NanoPac has been reconstituted it will be delivered to the clinic for use. Reconstitution will occur in the pharmacy at the clinical site, and if the reconstituted agent is not being delivered immediately the syringe may be stored according to the IFU until delivery. Each syringe must be labelled with the subject's ID and visit information for accountability purposes.

6.1.4 PREPARATION

Preparation of the study doses will be according to the IFU provided in the study kit.

A prescription will be provided for each subject detailing the subject ID, the volume of the lobe of the prostate containing the dominant lesion and the 20% calculation, and noting the cohort to which the subject is assigned. The prescription will also note the date and time required for administration, and this will be provided to the pharmacy at least 24 hours prior to administration time.

Once the drug has been reconstituted to the required dose (6, 10, or 15mg/mL, according to cohort assignment), the volume for use (being 20% of the lobe of the prostate with the dominant lesion) will be withdrawn from the vial into a syringe.

The syringe for administration will be labeled with the subject ID, the volume contained in the syringe as specified on the prescription, and the date and time of preparation.

6.1.5 DOSING AND ADMINISTRATION

Following successful completion of the screening period and after all eligibility requirements have been confirmed, the study agent will be administered via direct injection into the lobe of the prostate containing the dominant lesion four weeks prior to the scheduled prostatectomy. The dominant lesion is defined as the lesion with the highest Gleason score detected on biopsy prior to study enrollment. An enema will be performed 12 hours prior to the procedure to optimize ultrasound visualization.

The patient will be advised to report for the prostate NanoPac injection procedure with a full bladder. At the time of injection, a bladder scan will be performed to assess the bladder volume. If the volume is greater than 200mL, the injection procedure will be undertaken. If the volume is less than 200mL, the patient will be encouraged to drink water to increase the bladder volume to 200mL. A volume of 200mL is chosen to dilute any drug that can potentially extravasate into the bladder at the time of the prostate injection. The patient can have the option of a catheter being passed into the bladder to fill to the desired volume.

After the procedure the patient will be asked to void to empty the bladder and a post-void residual will be checked with the bladder scanner. If the volume in the bladder is greater than 150 mL, an in-and-out catheter will be performed to empty the bladder.

The patient will be placed in the lithotomy position with perineum exposed and scrotum elevated. Local anesthetic will be injected via a 22-gauge needle to perform a periprostatic nerve block. A Halyard 22G x 10” Quinke Point needle connected to the syringe containing the study agent will be inserted transrectally into the lobe of the prostate containing the dominant lesion under real-time MR-TRUS guidance. The needle will be retained in the rectum and reinserted into different ipsilateral regions of the prostate several times in a fanning pattern to infuse the lobe of the prostate with NanoPac, avoiding the midline area of the prostate adjacent to the urethra.

6.1.6 ROUTE OF ADMINISTRATION

NanoPac will be injected directly into the lobe of the prostate containing the dominant lesion.

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

NanoPac will be administered at concentrations of 6, 10, and 15 mg/mL in an injection volume of 20% of the lobe of the prostate containing the dominant lesion. The volume of the ipsilateral lobe of the prostate will be evaluated by ultrasound prior to study entry and this will be used for calculating the injection volume (Section 4.1).

Cohorts will be enrolled sequentially starting at the lowest concentration. Each cohort will start with planned minimum of three subjects, each receiving a single dose of the study agent. Dose escalation to the next cohort will proceed following review of data by the DSMB. Total prostate volume, ipsilateral lobe volume (50% of prostate volume), NanoPac dose and NanoPac injection volume will be documented in the Subject’s record.

Data from the three subjects in a cohort, including all DLT described in this section will be reviewed and evaluated by the DSMB to determine whether the dose received is considered safe and tolerable, and to determine whether dose escalation may occur. DLT information will be collected in an ongoing manner from the time of dosing until prostatectomy. DLTs considered to be serious adverse events (SAE) will be reported within 24 hours (Section 8.1.2). To determine dose escalation, the DSMB will review the data on the three subjects once they have completed the two-week follow-up visit, and will assess safety and tolerability based on the DSMB Charter. The DSMB will determine whether to:

(a) escalate to the next dose level cohort (no DLT);
(b) add three additional subjects to the current cohort (one DLT); or
(c) return to the previous (lower) dose cohort and expand by three subjects (greater than one DLT).

Included in the DSMB's review of the AE and general study data pertaining to safety there will be rules for non-escalation. Any adverse event that is considered related or possibly related to NanoPac is potentially a DLT. The definition of a DLT will be made by consensus by the Medical Monitor, Sponsor Medical Director, and Principal Investigator for AE; DLT are described in Sections 8.4.4 and 8.4.5 and include the following:

- Events which may occur and indicate possible systemic exposure, including anemia and neutropenia, alopecia, peripheral neuropathy, and prolonged periods of diarrhea, nausea and vomiting;
- Any Grade 2 toxicity lasting for ≥ one week;
- Any delay of scheduled prostatectomy due to study drug related toxicity;
- Symptomatic urine retention secondary to a study injection-related blockage of the prostatic urethra;
- ≥ Grade 3 febrile neutropenia (non-respondent to growth factors);
- Grade 4 neutropenia lasting 5 days;
- Grade 3 thrombocytopenia with clinically significant bleeding;
- Grade 4 thrombocytopenia;
- ≥ Grade 3 diarrhea and vomiting persisting after treatment with optimal anti-diarrheals or antiemetics;
- Severe pain (i.e., pain score ≥8/10) requiring opioid management, and which occurs when in the opinion of the Investigator all other procedural complications have been excluded, will also be considered a DLT.

The dose selected for further evaluation, defined as the highest dose with an acceptable safety and tolerability profile as determined by the DSMB, will enroll a total of 12 subjects. If one or fewer subjects in a six-subject cohort, or no subjects in a three-subject cohort at the highest dose, experience DLT, that cohort will be taken into the Dose Confirmation Phase. If greater than one subject in a six-subject cohort experience DLT, the previous dose will be taken into the Dose Confirmation Phase.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

As this study is evaluating one single dose of NanoPac in each subject, there will be no dose adjustment or modification in an individual subject.

6.1.9 DURATION OF THERAPY

A single administration of NanoPac is being injected directly into the lobe of the prostate containing the dominant lesion; therapy does not continue over an extended period.

6.1.10 TRACKING OF DOSE

Not applicable.

6.1.11 DEVICE SPECIFIC CONSIDERATIONS

Not applicable.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

The Investigator will maintain adequate records showing the receipt, dispensing, return, or other disposition of the study agent, including the date, quantity, batch or code number, and identification of patients (number, initials) who received study agent.

Accountability will be conducted on the vial packaging, the individual vials, and the syringes.

Under no circumstances will the Investigator supply clinical material to other Investigators or clinicians or allow the supplies to be used other than as directed by this protocol without the consent of the Sponsor.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

The following procedures and evaluations will be done as part of this study.

- Complete medical history to be completed, documented, and reviewed by the Investigator within 14 days prior to NanoPac administration, including review of previous medical records, demographics, and parity;
- Review and documentation of concomitant prescription and non-prescription medications;
- Review and documentation of diagnosis of adenocarcinoma of the prostate (including biopsy results, ultrasound results, and mpMRI results) and previous treatments including surgical and chemotherapeutic records. The biopsy results will be used to identify the dominant lesion. The ultrasound results will be used to determine prostate volume and volume of the lobe containing the dominant lesion. A copy of the pathology report confirming the diagnosis must be filed in the subject's study record;
- Comprehensive physical examination, including ECOG Performance Status assessment (Appendix A), and vital signs (blood pressure, heart rate, temperature, body weight and height);
- Sexual Health Inventory for Men (SHIM) [Appendix B], International Prostate Symptom Score (I-PSS) [Appendix C], and National Institutes of Health (NIH) Prostatic Symptoms Questionnaires [Appendix D] will be completed at screening and three days prior to prostatectomy;
- Sample collection and processing for clinical laboratory assessments at screening/baseline, 7 days after injection (hematology and biochemistry only) 14 and 21 days after injection, and immediately prior to prostatectomy as follows:
    - Sodium, potassium, chloride, carbon dioxide (CO_2), calcium, phosphorus, glucose, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), total bilirubin, direct bilirubin, aspartate

aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total protein, albumin, triglycerides, cholesterol, uric acid and calculated creatinine clearance;
    - Red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC) including differential, reticulocyte count, and platelet count;
    - Urinalysis including specific gravity, hydrogen ion concentration (pH), RBC, WBC, protein, and glucose;
    - Prothrombin time (PT) and activated partial thromboplastin time (PTT);
- Pharmacokinetic (PK) samples will be taken on Day 1 at 1, 2, 4, and 6 hours post-injection, and weekly until prostatectomy;
- Serum PSA will be determined prior to NanoPac injection and weekly in the interval between NanoPac injection and prostatectomy;
- Bladder scan prior to NanoPac injection to assist with visualization, and following voiding after the injection procedure to ensure minimal risk of retention;
- Imaging with mpMRI within 48 hours prior to prostatectomy;
- Direct injection of NanoPac into the prostate lobe containing the dominant lesion under MR-TRUS guidance;
- Ejaculate collection to determine presence of paclitaxel;
- Biopsy performed under anesthesia immediately prior to prostatectomy;
- Evaluation of excised prostate and lymph nodes after prostatectomy.

7.1.2 STANDARD OF CARE STUDY PROCEDURES

The subjects being enrolled to this study will be undergoing a scheduled prostatectomy. Routine work-up prior to surgery, and the surgery itself, are considered standard of care. Follow-up to surgery will be per standard of care.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Clinical laboratory assessments performed at screening/baseline are noted in Section 7.1.1. These tests will be conducted at the local Clinical Laboratory Improvement Amendments (CLIA) certified laboratory routinely used by the Investigator.

7.2.2 OTHER ASSAYS OR PROCEDURES

Pharmacokinetic (PK) samples will be taken on Day 1 at 1, 2, 4, and 6 hours post-injection, and weekly until prostatectomy. PK samples on Day 1 will allow for a 10-minute window around the samples.

The biopsy taken immediately prior to prostatectomy will look at the effects of NanoPac on the tumor cells and will be compared with the first biopsy taken prior to study participation which confirmed the presence of adenocarcinoma. The sample will be processed for histologic evaluation of the sites where tumor was previously identified and whether any tumor kill has been achieved.

Excised prostate and pelvic lymph nodes will be evaluated to determine presence of paclitaxel and presence of tumor cells.

Serum PSA will be determined prior to NanoPac injection and weekly during the interval between NanoPac injection and prostatectomy.

Imaging with ultrasound and mpMRI will have been conducted prior to study participation as part of the routine evaluation and confirmation of adenocarcinoma. These results will serve as the pre-treatment (or Baseline) data, prior to NanoPac injection; another study-specific mpMRI will then be conducted within 48 hours prior to prostatectomy, and ultrasound will be performed as part of the prostatectomy procedure.

In the interval between NanoPac injection and prostatectomy, starting one week after injection, ejaculate will be collected on a weekly basis and evaluated for presence of paclitaxel.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

PK samples will be drawn at the specified time/visit and stored frozen on-site until a cohort has completed all draws for analysis, at which time they will be batch-shipped to Covance Laboratories (Madison, WI) for analysis.

Following surgery, a representative section of the prostate and associated lymph nodes will be obtained from the excised tissue and will be immediately frozen and prepared for shipment to Frontage Laboratories (Exton, PA) for analysis of the presence of paclitaxel in the excised sample. The remainder of the excised prostate and lymph nodes will be routinely inspected in the operating room and sent (unfrozen) to Pathology for histological analysis.

Immediately prior to prostatectomy, a biopsy sample will be taken and sent to Pathology for histopathologic evaluation and comparison with the initial biopsy results obtained confirming the presence of adenocarcinoma.

Serum PSA samples will be obtained at the specified time/visit and will be sent to the local CLIA certified laboratory for analysis. Results will be sent to the Investigator for the source record.

Samples of ejaculate obtained at the time/visit specified will be snap frozen and stored on site until a cohort of subjects has completed, at which time all cohort samples will be shipped to Frontage Laboratories for analysis of the presence of paclitaxel. Ejaculate samples may be obtained at home the day prior to the clinic visit and stored in the freezer overnight; samples will be transported from home to the clinic in a cool bag on an ice block which will be provided at the prior clinic visit.

7.2.4 SPECIMEN SHIPMENT

Samples for paclitaxel assessment will be batch shipped, in a temperature-controlled environment, to Frontage labs when all samples for the cohort are available. Routine laboratory samples and PSA samples will be sent to the local laboratory upon collection. Tissue samples for histological evaluation will be sent to the local laboratory upon collection.

7.3 STUDY SCHEDULE

7.3.1 SCREENING

The following procedures and assessments must be completed, documented and reviewed by the Investigator during the screening period within 6 weeks prior to the scheduled prostatectomy, and therefore 14 days prior to the intended NanoPac injection:

- Written informed consent including comprehensive discussion of the study schedule, procedures, and subject protocol requirements;
- Complete medical history, including review of previous medical records, demographics and parity;
- Review and documentation of adenocarcinoma of the prostate diagnosis (including biopsy results, ultrasound results, and mpMRI results obtained within three months of consent) and previous treatments including surgical and chemotherapeutic records. The biopsy results will be used to identify the dominant lesion. The ultrasound results will be used to determine prostate volume and volume of the lobe containing the dominant lesion. A copy of the pathology report confirming the diagnosis must be filed in the subject's study record;
- Review and documentation of all concomitant prescription and non-prescription medications;
- Comprehensive physical examination, including ECOG Performance Status assessment (Appendix A), and vital signs (blood pressure, heart rate, temperature, body weight and height);
- SHIM, I-PSS, and NIH questionnaires (Appendices B-D);
- Sample collection and processing for clinical laboratory assessments:
    - Hematology, biochemistry, urinalysis, PT and PTT, and PSA levels
- Rectal swab will be taken and cultured to aid antibiotic prophylaxis selection for the NanoPac injection.
- Subject will be provided specific information and a prescription for the enema pack to be used twelve hours prior to the NanoPac dosing procedure at the next study visit.

7.3.2 ENROLLMENT/BASELINE

DAY 1 – Baseline and Treatment – Day of NanoPac Injection

The day of NanoPac injection (always considered Day 1) will be scheduled relative to prostatectomy, with NanoPac injection occurring four weeks (28 days) prior to the day planned for prostatectomy. However, the realities of medical practice (i.e. the availability of procedure rooms) may require that NanoPac injection and prostatectomy occur on different days of the week. For this reason, a window of two days either side (28 days prior to prostatectomy, plus or minus two days) is permitted for the Day 1/NanoPac injection visit. An extension beyond this window for NanoPac injection would require a waiver to be negotiated and obtained in advance from the Sponsor.

All subsequent follow-up study visits (Days 8, 15, and 22) will be scheduled relative to Day 1/NanoPac injection visit (**NOT prostatectomy)**, with a window of plus or minus one day permitted.

For example, if prostatectomy is scheduled for a Thursday, all clinic visits leading up to the prostatectomy would *ideally* be scheduled on the preceding Thursdays; however, the plus or minus two days window would permit the NanoPac injection to be scheduled on a Tuesday, Wednesday or Friday. Assuming the day of injection (Day 1) is a Tuesday, visits for the next three weeks would be scheduled to occur *ideally* on Tuesdays, but could be Monday or Wednesday (allowing for the window). The prostatectomy would occur, as scheduled, on Thursday and would be Day 31.

Following review of all test results from the screening visit, the following will be conducted on Day 1:

- Review of inclusion and exclusion criteria and determination of eligibility to proceed to treatment;
- Vital signs obtained;
- PSA sample to be obtained prior to dosing;

- Preparation for dosing;
    - Subject will have an enema 12 hours prior to dosing;
    - Subject will have a bladder scan prior to injection to assist with visualization and post-injection following voiding.
- Subject will receive NanoPac dose as described previously, according to the cohort allocation;
- PK Samples will be drawn at 1, 2, 4, and 6 hours post-dose. PK samples on Day 1 will allow for a 10-minute window around the samples;
- Concomitant medication will be reviewed and updated as necessary;
- AE will be collected from the time of dosing;
- Subject will be provided ciprofloxacin 500 mg to take twice a day for three days (or another antibiotic regimen as determined by the treating physician's discretion if resistant organism found on rectal swab culture) after NanoPac injection.

7.3.3 FOLLOW-UP

Follow-up study visits will be scheduled relative to Day 1/NanoPac injection, with a window of plus or minus one day permitted.

DAY 8

Seven days after dosing, the subject will return to the clinic for the following evaluations:

- Vital signs will be obtained;
- PK samples will be taken;
- Blood sample for hematology and biochemistry monitoring;
- AE will be recorded;
- Concomitant medication will be reviewed and updated as necessary;
- An ejaculate sample will be obtained;
- PSA sample will be obtained.

The subject will undergo any other evaluations determined necessary by the Investigator to ensure the subject's well-being.

DAYS 15 AND 22

At these weekly visits the following evaluations will be performed:

- Vital signs will be obtained;
- PK samples will be taken;
- Blood sample for hematology and biochemistry monitoring - Day 15 only;
- Urine sample for urinalysis - Day 15 only;
- AE will be recorded;
- Concomitant medication will be reviewed and updated as necessary;
- An ejaculate sample will be obtained;
- PSA samples will be obtained.

The subject will undergo any other evaluations determined necessary by the Investigator to ensure the subject's well-being.

DAY 29 – DAY OF SCHEDULED PROSTATECTOMY

Due to the window permitted for the Day 1/NanoPac injection visit, the day of prostatectomy injection may actually occur anytime between Day 27 and Day 31.

Subjects will be attending the facility within 48 hours of the prostatectomy procedure for a routine pre-operative work-up (anesthesiology assessment) as standard of care for the surgery. The following assessments may be performed during that visit or at any point within 48 hours of the surgery and will form part of the Day 29 data.

- SHIM, I-PSS, and NIH questionnaires will be completed;
- Ejaculate will be collected;
- MP-MRI will be performed;
- Full physical exam, including ECOG status.

The following should be performed on the day of surgery:

- Vital signs will be collected;
- Blood sample for hematology and biochemistry monitoring;
- Urine sample for urinalysis;
- PK samples will be taken;
- A serum sample will be drawn for PSA levels;
- Concomitant medications will be reviewed and updated as necessary, including post-surgical medications;
    - Surgical medications will be noted separately and a copy collected/retained for monitoring. This information will be available should it be required for further evaluation in the future; however, it will not be captured in the EDC as study medication;
- AE prior to surgery will be documented, and those occurring post-surgery will be documented and noted to be post-surgical events, some of which may be anticipated.

On the day of prostatectomy, after anesthesia has been administered, a biopsy will be performed. Immediately following biopsy, under the same anesthesia, subjects will have the scheduled prostatectomy and, following surgery, samples will be taken from the excised tissue (prostate and lymph nodes) for evaluation of presence of paclitaxel and for assessment of tumor cells, as described previously.

The subject will be followed as per standard of care during the post-surgical period; however, this data is not captured as part of the study.

7.3.4 FINAL STUDY VISIT

The final study visit will be upon discharge from hospital after prostatectomy. At this time the subject will exit the study with no further follow-up requirements unless there are ongoing AE not considered related to the surgery (see Section 8.3). Following the prostatectomy, the patient will return to standard of care with their follow up continued as recommended by their treating physician.

7.3.5 EARLY TERMINATION VISIT

In the event a subject is withdrawn they would, at minimum, undergo final study visit evaluations (End-of-Study evaluations) which include vital signs, AE collection and concomitant medication updates. If a subject is withdrawn at a routine study visit, all evaluations that would have been done at that study visit should be completed, as far as possible, and the least amount of information that would be captured are the vitals, AE, and concomitant medications.

7.3.6 UNSCHEDULED VISITS

Any unscheduled visits will be documented in the source documents, and any assessments and/or evaluations performed will be noted and reviewed. If the unscheduled visit occurs after dosing but prior to surgery, the following assessments should be conducted to monitor the ongoing safety of the subject:

- Vital signs will be obtained;
- AE will be recorded;
- Concomitant medication will be reviewed and updated as necessary.

The subject will undergo any other evaluations determined necessary by the Investigator to ensure the subject's well-being. If the Investigator deems it necessary for blood work to be done, this information will be filed in the source documents and be available if required at a later date, but laboratory results will not be transcribed into the EDC.

7.3.7 SCHEDULE OF EVENTS TABLE

	Screening Clinic Visit (Up to -14 days)	Day 1[13]	Injection-to-Surgery Interval[13]			Day 29[13] (Prostatectomy Surgery)		Hospital discharge
			Day 8	Day 15	Day 22	48-hour Window	Day Of Surgery	
Informed Consent	X							
History[1]	X							
Concomitant therapy	X	X	X	X	X		X	X[9]
Physical Exam	X					X		
ECOG[5]	X					X		
Questionnaires[10]	X					X		
Vital Signs	X	X	X	X	X		X	X
Hematology	X		X	X			X	
Biochemistry	X		X	X			X	
Urinalysis	X			X			X	
PSA	X	X	X	X	X		X	
Rectal Swab[8]	X							
PK Samples		X[7]	X	X	X		X	
Radiologic Assessment[2]						X		
Antibiotics[8]		X						
Enema[12]		X						
Bladder Scan		X						
NanoPac[6]		X						
Ejaculate collection			X	X	X	X		
Biopsy[11]							X	
Prostatectomy[4]							X	
Adverse Events[3]		X	X	X	X		X	X[9]
End of Study								X

1 History includes all events before initiation of NanoPac treatment.
2 Imaging assessment will utilize multiparametric MRI (mpMRI).
3 Adverse event determination will start immediately following initiation of study treatment.

[4] Procedure will include ultrasound evaluation at time of biopsy; following prostatectomy, excised prostate and pelvic lymph nodes will be evaluated to determine presence of paclitaxel as well as the volume of tumor cells.

[5] ECOG Performance Status Scale attached as Appendix A

[6] NanoPac will be administered by direct injection to the lobe of the prostate containing the dominant lesion with the aid of MR-TRUS and MRI

[7] PK Samples on Day 1 will be drawn at 1, 2, 4, and 6 hours post-dose. PK samples on Day 1 will allow for a 10-minute window around the samples.

[8] A rectal swab will be taken and cultured during screening to aid antibiotic prophylaxis. Subject will be provided ciprofloxacin 500 mg to take twice a day for three days (or another antibiotic regimen as determined by the treating physician's discretion if resistant organism found on rectal swab culture) after NanoPac injection.

[9] AE and concomitant medications at the end of study visit which are not considered due to the prostatectomy surgery will be followed; those considered due to the prostatectomy surgery (such as pain, nausea etc.) will not be captured nor followed

[10] Questionnaires include Sexual Health Inventory for Men (SHIM), International Prostate Symptom Score (I-PSS), and National Institutes of Health (NIH) Prostatitis Symptoms

[11] Biopsy will be performed immediately prior to prostatectomy under the same anesthesia.

[12] The evening prior to dosing or on the morning of dosing (12 hours prior to procedure) the subject will receive an enema to assist with the visualization when the imaging is performed.

[13] The day of NanoPac injection (Day 1) will be scheduled relative to prostatectomy, with NanoPac injection occurring four weeks (28 days) prior to the day planned for prostatectomy. However, a window of plus or minus 2 days (28 days prior to prostatectomy, plus or minus two days) is permitted for the Day 1/NanoPac injection visit. All subsequent study visits (Days 8, 15, and 22) will be scheduled relative to the day of NanoPac injection (NOT prostatectomy), with a window of plus or minus one day permitted. Because of the window around the day of injection, prostatectomy may actually fall anywhere between Day 27 and Day 31.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

Sponsor acknowledges that injection of NanoPac into the lobe of the prostate containing the dominant lesion may qualify as a sensitive procedure and as such should be mentioned in this section.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

All concomitant prescription medications taken during study participation will be recorded on the electronic case report forms (eCRFs). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the eCRF are concomitant prescription medications, over-the-counter medications, and non-prescription medications.

Although no interaction studies have been conducted using NanoPac, paclitaxel is metabolized by cytochrome P450 isozymes CYP2C8 and CYP3A4 (Taxol Package Insert). Thus, there is a potential for drug interactions with concomitantly administered substrates (e.g., repaglinide and rosiglitazone), inhibitors (e.g., gemfibrozil), and inducers (e.g., rifampin) of CYP2C8. There is also the potential for paclitaxel to interact pharmacokinetically with CYP3A4 substrates (e.g., midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, and triazolam), inhibitors (e.g., atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin), and inducers (e.g., rifampin and carbamazepine).

7.6 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.7 PROHIBITED MEDICATIONS, TREATMENTS, AND PROCEDURES

Use of concomitant chemotherapy (other than the protocol-specified agents), immunotherapy, or systemic use of hormonal therapy (such as GnRH analogs, antiandrogens, androgen receptor inhibitors, and 5-α reductase inhibitors) at any time prior to surgery is prohibited.

7.8 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Twelve hours prior to dosing the subject will receive an enema to assist with the visualization when the imaging is performed.

Subject will be instructed to take ciprofloxacin 500 mg twice a day for three days (or another antibiotic therapy as determined by the treating physician's discretion) after NanoPac injection as prophylactic antibiotic therapy.

7.9 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.10 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Not applicable.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

Safety assessments being conducted in this study include:

- AE, collected at all study visits from the time of dosing;
- Changes in concomitant medications;
- Findings from physical examinations;
- Changes in vital signs.

Safety will be reviewed by the Medical Monitor in an ongoing manner via the EDC system, and details will be confirmed at routine on-site monitoring visits. Additionally, DSMB assessments of safety and tolerability will be conducted after every three subjects are dosed and have completed prostatectomy post-NanoPac injection (or more frequently if deemed necessary) prior to any dose escalation occurring, as detailed in Section 6.1.7.

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended change in structure, function, signs, or symptoms temporally associated with the use of a medicinal product, whether or not related to the product. Undesirable changes in laboratory values should not be considered AE unless they are considered symptomatic of a clinical condition or diagnosis, are evaluated as clinically significant, or require therapy. Worsening of a pre-existing condition is also considered an AE, as is the discovery of an abnormal finding during physical exam that was not included in the medical history. Clinical conditions attributable to disease progression will be considered AE and reported on the eCRF.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A Serious Adverse Event (SAE) is any adverse event that meets at least one of following criteria:

1) Is fatal;
2) Is life-threatening, meaning the patient was, in the view of the Investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more serious form or progressed, might have caused death;
3) Is a persistent or significant disability or incapacity;
4) Requires or prolongs inpatient hospitalization. Inpatient hospitalization will be considered a hospitalization that is longer than 24 hours, or a hospitalization that requires an intervention to treat emergent symptomatology (non-diagnostic);
5) Is a congenital anomaly or birth defect;
6) Other important medical events may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes as listed in #1-5 in this definition.

8.1.3 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

As this is a Phase IIa study, all unanticipated problems will be captured as either AE or SAE and will be defined and reported accordingly.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

Signs and symptoms will be graded by the Investigator as mild, moderate, severe, life-threatening, or fatal according to the following definitions:

- **Mild:** Causing no limitation of usual activity
- **Moderate:** Causing some limitations of usual activities
- **Severe:** Causing inability to carry out usual activities
- **Life-Threatening:** Patient was at immediate risk of death from the event
- **Fatal:** Death related to the event.

Toxicities should be evaluated according to the NCI CTCAE, version 4.0.
https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Toxicity grades should be recorded as: 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Life-Threatening, 5 = Fatal.

8.2.2 RELATIONSHIP TO STUDY AGENT

Events will be considered drug-related if classified by the Investigator as possible, probable, or definite. Association of events to the study agent will be made using the following definitions:

- No relationship to study agent: the event is not associated with study agent.
- Possibly related to study agent: the event follows a reasonable temporal association with the study agent administration, however could have been produced by the patient's clinical condition or other therapy.
- Probably related to study agent: the event follows a) a reasonable temporal association with the study agent administration, but b) abates upon discontinuation of study agent and c) cannot be explained by the patient's clinical condition or other therapy.
- Definitely related to study agent: the event: a) follows a reasonable temporal association with the study agent administration, but b) abates upon discontinuation of study agent, c) cannot be explained by the patient's clinical condition or other therapy, and d) reappears on re-exposure to study agent.

8.2.3 EXPECTEDNESS

The definition of expectedness is related to the study agent specifically. An event may be unexpected in the subject but that in itself does not qualify as unexpected; review against information available and provided for the study agent is what will determine expectedness.

Sponsor will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent in the protocol and within the Investigator's Brochure.

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

AE should be followed until resolved, stabilized or, if ongoing at End-of-Study (study-related AE only), for a minimum of 30 days following the termination of the subject's participation from the study for any reason. Those AE considered to have occurred due to the surgery do not form part of the study, and therefore will be followed per routine clinical practice. Subjects will be required to spontaneously report any AE. Study personnel will ask open-ended questions to obtain information about AE at every visit. Date and time of onset and resolution (if applicable) of the AE will be documented.

All AE and SAE must be followed until the event resolves or, in the opinion of the Investigator, becomes stable. Hospitalization of patients for the scheduled prostatectomy will not be considered an SAE.

The Sponsor will report any serious, unexpected, and drug-related AE to applicable regulatory agencies and make these reports available to the investigative sites. The Investigator must promptly inform the IRB of such events and retain a copy of the notification in the site's regulatory binder.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

All AE (whether or not attributable to the study agent) occurring during the study observed by the Investigator or reported by the subject will be recorded on the eCRF. The following information will be recorded for all AE:

- Name of condition/diagnosis/description
- Onset and resolution dates
- Severity
- Relationship to study agent
- Action taken
- Seriousness

8.4.2 SERIOUS ADVERSE EVENT REPORTING

All SAE, including death, due to any cause which occurs during this study between the period of dose administration and surgical prostatectomy (occurring 28 days following dose), whether or not expected and regardless of relationship to study agent, must be reported to the Medical Monitor immediately upon discovery of the event, using the SAE reporting form, by fax and, if necessary, by phone to:

Dr. Antony Verco
Medical Monitor
US Biotest, Inc.
Email: tony.verco@usbiotest.com
Phone: 805-762-4615
Fax: 805-980-4196

24-hour Emergency Contacts:	Gere diZerega, MD Medical Director 805-630-2800	or	Antony Verco, MD Medical Monitor 805-762-4615

The Sponsor will advise the Investigator regarding the nature of any further information or documentation that is required. The Investigator should provide the following documentation at the time of notification if available:

- SAE Report Form;
- Concomitant and support medication pages;
- Relevant diagnostic reports;
- Relevant laboratory reports;
- Admission Notes;
- Hospital discharge summary (when available).

8.4.3 UNANTICIPATED PROBLEM REPORTING

Incidents or events that occur during the conduct of the study and meet the criteria for an AE or SAE will be captured in the source documents and in the EDC, and in the case of an SAE, also on the formal reporting form designed to capture the required information. Reporting of these events will be in accordance with the rules around AE and SAE reporting described in the protocol, including notification of the IRB and/or the FDA as required.

8.4.4 EVENTS OF SPECIAL INTEREST

Subjects will receive a single administration of NanoPac and therefore any event seen as a result of the study agent will be managed according to best/standard practices.

Events that are common when performing prostate biopsy will be noted. These include pain (which can be persistent and require narcotics) and dysuria (which may require analgesia); complications following prostate biopsy include urinary tract infection (UTI), per rectal bleeding, hematuria, hematospermia, and urinary retention.

AE of special interest that occur due to the procedure and/or are related to the study agent will be documented in the subject's source records and within the electronic database, and may include the following:

- Anaphylaxis/allergic reaction – This could present as a spectrum of symptoms from an urticarial rash to cardiovascular and respiratory compromise. These effects would manifest immediately.
- Urinary retention – Patient unable to void and requires catheterization. The decision to catheterize depends on individual patient bladder capacity and abdominal discomfort. Interim data from a toxicokinetic study of intraprostatic injection in rats indicated there was some evidence of swelling of the prostate and resultant urinary outflow obstruction. The injection volume in the proposed Phase IIa clinical study has been limited to 20% of the ipsilateral lobe of the prostate specifically in order to prevent urinary outflow obstruction; however, it could still occur and would be of particular interest.
- Urosepsis – Urinary tract infection requiring hospitalization and intravenous antibiotics to monitor and stabilize hemodynamics. This would be considered a SAE, as it requires intervention.
- Gross Hematuria – Passing large volumes of clots that impede urinary flow. The patient could have intermittent flow or progress to urinary retention. For this situation, bladder catheterization and irrigation will be instituted.

- Perineal pain – Severe pain rated greater than 8/10 on a pain score assessment that requires opioid analgesia.
- Per-rectal bleeding – Requiring surgical intervention to stem the bleeding after injection. This would be considered a SAE as it requires intervention.

Of particular interest will be signs of systemic toxicity due to paclitaxel exposure. This is not expected and is unlikely due to the mode of administration and dose levels; however, if such signs occur they will be captured as events of special interest. Events which may occur and indicate possible systemic exposure include anemia and neutropenia, alopecia, peripheral neuropathy, and prolonged periods (3 or more days) of diarrhea, nausea and vomiting.

8.4.5 DOSE LIMITING TOXICITY

Throughout the study, particular attention will be paid to the possibility of DLT, and specific reference to these will be included in the DSMB Charter for the Board's review and consideration prior to dose escalation. DLT information will be collected in an ongoing manner from the time of dosing until prostatectomy. DLTs considered to be SAE (Section 8.1.2) will be reported within 24 hours.

Any AE that is considered related or possibly related to NanoPac is potentially a DLT. The definition of a DLT will be made by consensus by the Medical Monitor, Sponsor Medical Director, and Principal Investigator for AE. DLT include the following:

- Events which may occur and indicate possible systemic exposure, including anemia and neutropenia, alopecia, peripheral neuropathy, and prolonged periods of diarrhea, nausea and vomiting;
- Any Grade 2 toxicity lasting for ≥ one week;
- Any delay of scheduled prostatectomy due to study drug related toxicity;
- Symptomatic urine retention secondary to a study injection-related blockage of the prostatic urethra;
- ≥ Grade 3 febrile neutropenia (non-respondent to growth factors);
- Grade 4 neutropenia lasting 5 days;
- Grade 3 thrombocytopenia with clinically significant bleeding;
- Grade 4 thrombocytopenia;
- ≥ Grade 3 diarrhea and vomiting persisting after treatment with optimal anti-diarrheals or antiemetics;
- Severe pain (i.e., pain score ≥8/10) requiring opioid management, and which occurs when in the opinion of the Investigator all other procedural complications have been excluded, will also be considered a DLT.

8.4.6 REPORTING OF PREGNANCY

If sexually active, subjects must use double condoms from time of NanoPac injection until after prostatectomy.

Any pregnancy occurring in a sexual partner while a subject is in the study period between NanoPac injection and prostatectomy must be reported to US Biotest as soon as the Investigator is aware of it. The pregnancy will not be considered an SAE; however, information on these pregnancies will be collected and followed for the outcome of the pregnancy and the health of the newborn.

Any pregnancy complication or premature terminations including miscarriage, spontaneous abortion, or elective termination of a pregnancy for medical reasons will be reported as an SAE, as described in Section 8.4.2. Should the pregnancy result in a congenital anomaly or birth defect, a separate SAE report must be submitted.

Furthermore, all neonatal deaths that occur within 30 days of the birth should be reported as SAE, without regard to causality. In addition, any infant death that occurs after the 30-day reporting period that the Investigator suspects is related to the in utero exposure to the study agent should also be reported.

8.5 STUDY HALTING RULES

This study is a Phase IIa dose escalation study, and dose escalation will be determined following review of all safety and tolerability data of a cohort by the DSMB. Following review, the study may be terminated.

The DSMB may determine that the second set of three subjects should be dosed and complete at the same dose level as a current group (to obtain a cohort of 6) to provide additional safety and/or tolerability information needed in order to permit dose escalation in the next cohort; they may also determine that it is acceptable to proceed with an increased dose in the first three subjects of the next cohort; or they may determine that the safety and tolerability profiles are not acceptable and may stop the study.

The Sponsor is responsible for notifying the FDA of any temporary halts to the study or when a study is terminated; the Investigator will be required to notify the IRB accordingly.

8.6 SAFETY OVERSIGHT

Safety will be overseen by the Data Safety Monitoring Board (DSMB). A detailed DSMB Charter will be drawn up prior to study start and the Board will consist of at least three members, one of whom will be the study Medical Monitor.

All subject study data will be captured in an EDC system, allowing real-time access to ongoing safety and tolerability data. The Medical Monitor will review the data for each subject entered to the database on a regular basis throughout the study. In the event the Medical Monitor has any concerns or sees any safety trends emerging during his ongoing reviews, he will bring it to the immediate attention of the Medical Director (and the Principal Investigators, as appropriate).

Upon completion of a cohort, and prior to dose escalation proceeding, the DSMB will convene to review the cohort data. A report will be generated outlining any safety concerns from the data available for review in the EDC and from data tables generated from the EDC for this specific purpose. This review will take place prior to proceeding with either addition of more subjects to a current cohort or proceeding to dose escalate in a new cohort.

During the DSMB review, members will review all safety data as available in the EDC, provided as reports generated directly from the EDC system and provided by the Data Management group. Particular emphasis will be placed on the events of special interest, as outlined in Section 8.4.4, and on events which may constitute dose-limiting toxicities as outlined in Section 6.1.7.

9 CLINICAL MONITORING

US Biotest monitors, or monitors designated by US Biotest, will conduct scheduled site visits to the investigational center for the purposes of monitoring the study. The Investigator agrees to allow these monitors, and other authorized Sponsor personnel or designees, access to the patient's medical records, regulatory binder, study binder, eCRFs, and source documents as needed to assure the conduct of the study is within compliance. In addition, the FDA or other government agencies may request an inspection following notification to the site. In

such an event, the Investigator agrees to notify the Sponsor immediately of the request, and will allow Sponsor and inspectors to review records.

US Biotest will conduct a site initiation visit to provide the Investigator and their staff with a comprehensive overview of the protocol and study procedures and to review mutual obligations and requirements of regulatory authorities. A regulatory file or binder containing required documentation will be kept at the site for reference and inspection.

Routine monitoring visits will be made to assure compliance with the study protocol, to review and collect the patient's eCRF and compare with source documents, to ensure adequate records of clinical supplies are maintained, and to assess the continued suitability of the investigational site. On completion of the study, the monitor will make a final assessment of the conduct of the study and inventory all clinical supplies to be returned to US Biotest.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

A formal Statistical Analysis Plan (SAP) will be prepared for this trial, and the SAP will be signed off prior to study database lock.

10.2 STATISTICAL HYPOTHESES

No inferential analyses are proposed, thus no hypotheses are stated.

10.3 ANALYSIS DATASETS

All subjects who receive treatment will be included in the outcome presentations.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

In this Phase IIa dose escalation trial, the focus will be on providing descriptive statistical summaries including tables and graphs for each of the dose groups. The clinical/medical review of these data will determine whether there are any issues with toxicity that could be associated with dose and whether there is any effect of the treatments on the prostate tumor.

10.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary objective of this study is to evaluate the safety and tolerability of three doses of NanoPac (concentrations of 6, 10, and 15 mg/mL) injected directly into the lobe of the prostate with the dominant lesion in patients diagnosed with prostate cancer.

The adverse events reported will be coded in MedDRA and summarized by system organ class and preferred term for each of the dose groups. Where possible and relevant, these will also be subset temporally by date and time of onset (e.g., first 24 hours, up to Day 7); the details of the timeframes will be established medically and presented

in the SAP. Events reported as Grade 3 or greater on the ECOG scale (Appendix A) and those that lead to trial discontinuation will be noted. Any serious AE and any deaths will be summarized separately.

The vital signs raw data, collected at each visit, and changes from Day 1 will be tabulated and listed.

The laboratory analyses will be presented in summary tables with changes from Day 1 to Days 15 and 29. By applying the normal ranges (high, normal, and low) shift tables will be generated. Values which are noted by the Investigator to be abnormal and clinically relevant will be summarized separately as will any analytes where the shift in category is greater than two (e.g., high to low or low to high). The SAP may capture medically relevant changes (e.g., 3 x the normal range) and analytes which meet this criterion will also be presented separately.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

To address the secondary objective of the concentration of paclitaxel in the systemic circulation post-injection and prior to prostatectomy, the pharmacokinetic (PK) samples taken on Day 1 at 1, 2, 4, and 6 hours post-injection,and weekly until prostatectomy will be presented (at a minimum) by individual listings. If some concentration data is above the detectable limit and can be reported numerically, this data will be tabulated and graphed for individuals and by dose group. This will be supported by the ejaculate paclitaxel concentration data for Days 8, 15, 22, and 29, which will be tabulated as mean values for each of the dose groups.

The presence of paclitaxel in the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy will provide insight into the tumor environment.

The tumor response (change in image on mpMRI) will employ the radiological assessments at screening and pre-prostatectomy, and will be summarized for the mpMRI. Where possible, changes between Screening and the pre-prostatectomy assessments will be highlighted with change scores for continuous data and shift tables for categorical data for the dose groups. The histological evaluation of the biopsies, taken prior to screening confirming adenocarcinoma and prior to prostatectomy, will be summarized and treated in the same manner as the mpMRI data.

The presence of tumor cells in the tumor within the prostate and, if tumor is present, the volume of tumor cells, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy will be tabulated for each of the dose groups.

10.4.4 SAFETY ANALYSES

All safety analyses will be presented as part of the primary endpoint analysis.

10.4.5 ADHERENCE AND RETENTION ANALYSES

All subjects who are enrolled and treated in the trial will be accounted for. Subjects terminating early will be noted and the reasons provided.

10.4.6 BASELINE DESCRIPTIVE STATISTICS

Complete demographic, medical (coded in MedDRA), and disease history will be summarized for each of the dose groups.

10.4.7 PLANNED INTERIM ANALYSES

An interim analysis is not planned

10.4.7.1 SAFETY REVIEW

Not applicable.

10.4.7.2 EFFICACY REVIEW

Although this study is not designed to evaluate efficacy, there are several assessments which will provide some preliminary efficacy information, and details are included in 10.4.3. above.

In addition, PSA levels collected at screening, as well as Days 1, 8, 15, 22, and 29 will be presented graphically and summarized by dose group as raw values and changes from Day 1 to determine whether the PSA levels change with time and dose group. The ejaculate paclitaxel data for Days 8, 15 and 22 and pre-prostatectomy will be tabulated as present or absent for each of the dose groups.

In addition, one of the purposes of doing the biopsy immediately prior to prostatectomy is to provide information on whether the biopsy can accurately portray the effects of the drug when compared to the pre-screening biopsy, so that in the future if the drug provides any efficacy this information will be able to be obtained prior to a subject requiring radical prostatectomy.

10.4.8 ADDITIONAL SUB-GROUP ANALYSES

Not applicable.

10.4.9 MULTIPLE COMPARISON/MULTIPLICITY

Not applicable.

10.4.10 TABULATION OF INDIVIDUAL RESPONSE DATA

All data collected in the trial and any calculated outcomes derived from this data will, at a minimum, be listed with the dose group, subject identifier and a timepoint, if relevant. The organization of the listings will support the writing of the clinical study report (CSR) as outlined in the ICH E3 guidelines.

10.4.11 EXPLORATORY ANALYSES

Not applicable.

10.4.12 CONCOMITANT MEDICATION

All medication taken during the trial will be, at a minimum, listed with the study date start and stop. For this small clinical trial, the medications will not be coded using the World Health Organization (WHO) Drug Dictionary.

10.5 SAMPLE SIZE

There is no formal sample size calculation for this Phase IIa safety study. However, to provide a reference for the ongoing safety review of each cohort and the possible expansion of a cohort with safety concerns, nQuery Advisor (Version 6) employing the procedure "confidence interval for the probability of observing a rare event" determined that, for an event with an occurrence rate of 0.33, the probability of detecting it with 3 subjects is 69.9% vs 91.0% for 6 subjects, and for an event rate of 0.05 the probability of detecting the event was 14.3% and 26.5% for 3 and 6 subjects respectively. The rationale to expand the final cohort to 12 subjects, from either 3 or 6 subjects, was based on the "reasonable gain" in detection rate that each additional subject would provide in this early phase exploratory trial.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Not applicable.

10.6.2 EVALUATION OF SUCCESS OF BLINDING

Not applicable.

10.6.3 BREAKING THE STUDY BLIND/PARTICIPANT CODE

Not applicable.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

An eCRF is required and must be completed for each consenting and enrolled subject by qualified and authorized personnel. Subjects who fail the screening assessments will not have an eCRF. All data in the eCRF must reflect the corresponding source documents. Any corrections to entries made on the eCRF must be documented in a valid audit trail. Only data required by the protocol for the purposes of the study should be collected within the EDC.

The Investigator must maintain adequate and accurate source documents on which the eCRFs for each patient are based. They will be separate and distinct from the eCRFs. These records should include detailed notes on:

- Medical history prior to the patient's involvement in the study;
- Date of informed consent;
- Basic identifying information that links the patient's medical record with the eCRFs;
- Results of all diagnostic tests performed, diagnoses made, therapy provided, and any other data on the condition of the patient;
- Medical condition during the patient's involvement in the study;
- All AE;
- Patient's exposure to the study agent;
- Patient's exposure to any concomitant therapy;
- All relevant observations and data on the condition of the patient throughout the trial;
- Justification for all entries in the patient's eCRF.

12 QUALITY ASSURANCE AND QUALITY CONTROL

Data required by the protocol will be collected and entered into a validated data management system that is compliant with all regulatory requirements. The eCRF is an electronic document designed to record all of the protocol-required information to be reported to the Sponsor on each study subject.

Data recording must follow the instructions described in the CRF Completion Guidelines. The Principal Investigator has ultimate responsibility for the collection and reporting of all clinical data entered on the eCRF. The PI or designee, as identified on Form FDA 1572, must electronically sign the completed eCRF for each participating subject to attest to its accuracy, authenticity, and completeness.

The EDC application being used in this study is TrialMaster® version 4.2.1 from OmniComm Systems. TrialMaster studies are hosted from a state-of-the-art data center with rigorous physical and electronic security. All data is backed up daily to Iron Mountain, in Ohio and is also backed up to a hurricane-proof bunker in Fort Lauderdale, Florida. OmniComm has achieved certification with European “Safe Harbor” regulations, meaning that all necessary measures are in place to protect patient confidentiality even with the data being stored in a US data center. The data management and statistical CRO, McDougall Scientific Ltd., ensures that the development of the eCRF follows their SOPs which are based on the Systems Development Life Cycle (SDLC) methodology. Access to the system is restricted by username and password; these are controlled by the Data Management CRO. All personnel using the system will be trained and the training documented. All changes to the database are recorded in an audit trail. The database will be locked when all outstanding queries have been addressed, all agreed to data is marked as source verified, and the PI has signed off on the eCRF contents.

All software applications used in the collection of data will be properly validated following standard computer system validation that is compliant with all regulatory requirements.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki. Sponsor and Investigator will comply with their responsibilities as defined in 21 CFR 312.50-312.70.

13.2 INSTITUTIONAL REVIEW BOARD

Before the start of the study, the study protocol, informed consent form and/or other appropriate documents will be submitted to the IRB and/or the authorities in accordance with local legal requirements. It is the responsibility of the Investigator to assure that all aspects of the IRB review are conducted in accordance with current regulations. US Biotest and the Investigator must inform each other in writing that all ethical and legal requirements have been met before the first patient is enrolled in the study.

Amendments to the protocol will be subject to the same requirements as the original protocol. All changes to the consent form will be IRB-approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Subjects being considered for participation in this study will be provided an informed consent form (ICF) to read and sign before being permitted to participate. The ICF will describe the study agent and any prior findings from previous studies; study procedures including the timing of study clinic visits and their responsibilities to adhere to those timelines; any risks which may be associated with the study agent or the procedures being carried out in the study; and all other items required under 21 CFR Part 50.25.

Subjects will be required to provide signed consent prior to the performance of any study-related procedures. The Investigator is required to document the process for obtaining informed consent in the source notes.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

The Investigator will obtain informed consent from each patient enrolled in the study, in accordance with the U.S. Food and Drug Administration (FDA) regulations 21 CFR 50.20 - 50.27 and the laws and regulations of the state in which the investigation is being conducted. The IRB must approve ICF to be used by the Investigator. The Investigator will provide the Sponsor with a copy of the written approval generated by the IRB or Ethics Committee before the Investigator will be permitted to enroll subjects into the study.

It is the responsibility of the Investigator to ensure that informed consent is obtained from the patient or his/her guardian or legal representative before any activity or treatment is undertaken which is not part of routine care. This includes, but is not limited to, the performance of diagnostic or therapeutic procedures and the administration of the first dose of study agent. The draft version of the informed consent document will be modified by each site and reviewed and approved in writing by US Biotest prior to submission to the IRB.

Should a protocol amendment be made, the subject consent form may be revised to reflect the changes of the protocol. If the consent form is revised, it is the responsibility of the Investigator to ensure that an amended consent is approved by the IRB and signed by all subjects currently on study as well as those subsequently entered in the study.

The Investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF. The original, signed informed consent document must be maintained on file at the study site and be made available for review during monitoring visits and site audits.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

All local legal requirements regarding data protection will be enforced. All study findings and documents will be regarded as confidential. The Investigator and members of his/her research team must not disclose such information without prior written approval from US Biotest.

The anonymity of participating subjects must be maintained to the extent required by law. Throughout documentation and evaluation, the subjects will be identified on eCRFs and other documents submitted to US Biotest by their initials, birth date, and patient number. The subjects will be told that all study findings will be

stored and handled in strictest confidence, according to legal requirements, but will be informed that authorized research Investigators and agents of the FDA, the National Cancer Institute, and authorized personnel of US Biotest have the right to inspect their medical records.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

Samples and data collected under this protocol are specifically for use in the evaluation and analyses being conducted in the study. Samples will not be available for purposes other than indicated within this protocol, and no genetic testing will be performed.

Access to stored samples will be limited to personnel authorized to have access at the site prior to shipping to the laboratories for analysis/assessment. Samples will be stored using codes assigned by the Sponsor or as required by the clinical laboratories.

Samples will only be retained until analysis is complete, following which samples will be disposed of according to the laboratory SOPs. No samples will be retained for any future use.

Data will be kept in password-protected computers. Only Investigators and those delegated responsibility on the Delegation of Authority Log will have access to the samples and data.

13.5 FUTURE USE OF STORED SPECIMENS

Not applicable.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Source documents will be maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents. Any discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

The EDC application being used in this study is TrialMaster® version 4.2.1 from OmniComm Systems. TrialMaster studies are hosted from a state-of-the-art data center with rigorous physical and electronic security. All data is backed up daily to Iron Mountain, in Ohio and is also backed up to a hurricane-proof bunker in Fort Lauderdale, Florida. OmniComm has achieved certification with European "Safe Harbor" regulations, meaning that all

necessary measures are in place to protect patient confidentiality even with the data being stored in a US data center. The data management and statistical CRO, McDougall Scientific Ltd., ensures that the development of the eCRF follows their SOPs which are based on the Systems Development Life Cycle (SDLC) methodology. Access to the system is restricted by username and password; these are controlled by the Data Management CRO. All personnel using the system will be trained and the training documented. All changes to the database are recorded in an audit trail. The database will be locked when all outstanding queries have been addressed, all agreed to data is marked as source verified, and the PI has signed off on the eCRF contents.

Data recording must follow the instructions described in the eCRF Completion Guidelines. The PI has ultimate responsibility for the collection and reporting of all clinical data entered on the eCRF. The PI or designee, as identified on the Delegation of Responsibilities Log (and included on Form FDA 1572), must electronically sign the completed eCRF to attest to their accuracy, authenticity, and completeness.

The database will be locked when all outstanding queries have been addressed, all agreed to data is marked as source verified, and the PI has signed off on the eCRF contents.

14.2 STUDY RECORDS RETENTION

The Investigator must retain a copy of all study documents in accordance with the FDA or local regulations, whichever are the more stringent.

The Investigator must maintain study documents:

- For a minimum of two years following the date the marketing application (NDA) is approved for the indication for which the drug was investigated for;
- For a minimum of two years following the release date of the final report, if no marketing application is to be filed, or if the marketing application is not approved for the indication of which the drug was investigated or is discontinued and the FDA has been notified; or,
- For a minimum of 15 years after the completion or discontinuation of the study to be filed in support of the registration in the European Union.

If the Investigator relocates, retires or withdraws from the study for any reason, the study records may be transferred to an acceptable designee, such as another Investigator, another institution, or the Sponsor. The Investigator must obtain the Sponsor's written permission before transferring or disposing of any records.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), FDA or IRB requirements. The noncompliance may be either on the part of the participant, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5: Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
- 5.1: Quality Assurance and Quality Control, Section 5.1.1
- 5.20: Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations as soon as possible after occurrence and identification of the protocol deviation. All deviations must be addressed in study source documents, reported to the Sponsor and to the data Management group.

The noncompliance may be either on the part of the participant, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

Protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

Serious non-compliance on the part of the site, and an inability of the Sponsor to bring the site back into compliance, will be reported to the FDA in accordance with their requirements.

14.4 PUBLICATION AND DATA SHARING POLICY

By signing the study protocol, the Investigator agrees with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement. The Sponsor will prepare an integrated clinical/statistical report. Publication/presentation of data is not allowed without explicit permission from US Biotest. Submission of data for publication/presentation will be coordinated and approved by US Biotest in collaboration with the Investigator.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and AE. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Data entered to the ClinicalTrials.gov website will be in accordance with the FDA requirements for this registration and for publication of study results on that site.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

The study will be overseen by the Study Manager who will be responsible, together with the Investigator, for tracking enrollment, timelines, and deliverables, and other study-related performance.

All questions regarding the enrollment of subjects, regulatory requirements for the conduct of the study, safety reporting, or study conduct should be addressed to the Study Manager or Site Monitor designated by the Sponsor. Contact information for the Sponsor is provided near the beginning of this protocol and will be provided to the Investigator in separate study documents.

16 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical and therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. As required by the FDA, a Financial Disclosure Form will be completed by each person noted on the FDA Form 1572 for this study at the site, the original will be filed in the TMF, and a copy will remain in the site's regulatory binder.

17 LIABILITY AND INSURANCE

The Sponsor will take out reasonable third-party liability insurance coverage in accordance with all local legal requirements. This insurance will cover all parties involved in the trial including, but not necessarily limited to, the Principal Investigator, clinical trial site, and subjects.

18 LITERATURE REFERENCES

Axiak-Bechtel SM, Kumar SR, Dank KK, Clarkson NA, Selting KA, Bryan JN, Rosol TJ, et al. Nanoparticulate paclitaxel demonstrates antitumor activity in PC3 and Ace-1 aggressive prostate cancer cell lines. Invest New Drugs. 2013;31:1609-1615.

Belldegrun A, Tso AL, Zisman A, Naitoh J, Said J, Pantuck A, et al. Interleukin 2 gene therapy for prostate cancer: Phase I clinical trial and basic biology. Human Gene Therapy. 2001 May 20;12:883-892.

Berry WR, Hathorn JW, Dakhil SR, Loesch DM, Jackson DV, Gregurich MA, et al. Phase II randomized trial of weekly paclitaxel with or without estramustine phosphate in progressive, metastatic, hormone-refractory prostate cancer. Clin Prostate Cancer. 2004 Sept;3(2):104-111.

DeWeese TL, van der Poel H, Li S, Mikhak B, Drew R, Goemann M et al. A Phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. Cancer Res. 2001;61:7464-7472.

Finkelstein SE, Rodriguez F, Dunn M, Farmello MJ, Smilee R, Janssen W, et al. Serial assessment of lymphocytes and apoptosis in the prostate during coordinated intraprostatic dendritic cell injection and radiotherapy. Immunotherapy. 2012 April;4(4):373-382.

Freytag SO, Khil M, Stricker H, Peabody J, Menon M, DePeralta-Venturina M, et al. Phase I study of replication-competent adenovirus-mediated double suicide gene therapy for the treatment of locally recurrent prostate cancer. Cancer Res. 2002;62:4968-4976.

Fujita R, Teh BS, Timme TL, Mai WY, Satoh T, Kusaka N, et al. Sustained long-term immune responses after in situ gene therapy combined with radiotherapy and horomonal therapy in prostate cancer patients. Int J Radiat Oncol Biol Phys. 2006;65(1):84-90.

Gulley JL, Heery CR, Madan RA, Walter BA, Merino MJ, Dahut WL. Phase I study of intraprostatic vaccine administration in men with locally recurrent or progressive prostate cancer. Cancer Immonl Immunother. 2013;62(1):1521-1531.

Hudes GR, Nathan FE, Khater C, Greenberg R, Gomella L, Stern C, et al. Paclitaxel plus estramustine in metastatic hormone-refractory prostate cancer. Sem Oncol. 1995;22(5)(Suppl 12):41-45.

Hudes GR, Nathan F, Khater C, Haas N, Cornfield M, Giantonio B, Greenberg R, Gomella L, Litwin S, Ross E, Roethke S, McAleer C. Phase II trial of 96-hour paclitaxel plus oral estramustine phosphate in metastatic hormone-refractory prostate cancer. J Clin Oncol. 1997;15(9):3156-3163.

Hussain A, Wu Y, Mirmiran A, DiBiase S, Goloubeva O, Bridges B, et al. Long-term follow-up of a prospective trial of trimodality therapy of weekly paclitaxel, radiation, and androgen deprivation in high-risk prostate cancer with or without prior prostatectomy. Int J Radiat Oncol Biol Phys. 2012;82(1):167-174.

Johannsen M, Gneveckow U, Taymoorian K, Thiesen B, Waldofner N, Scholz R et al. Morbidity and quality of life during thermotherapy using magnetic nanoparticles in locally recurrent prostate cancer: Results of a prospective phase I trial. Int J Hyperthermia. 2007;23(3):315-323.

Johannsen M, Gneveckow U, Thiesen B, Taymoorian K, Cho CH, Waldofner N, et al. Thermotherapy of prostate cancer using magnetic nanoparticles: Feasibility, imaging, and three-dimensional temperature distribution. Eur Urol. 2007;52(6):1653-1561.

Kramer G, Steiner GE, Sokol P, Handisurya A, Klingler HC, Maier U, et al. Local intratumoral tumor necrosis factor-α and systemic IFN- α2b in patients with locally advanced prostate cancer. J Interferon Cytokin Res. 2001;21:475-484.

Marshall S, Taneja S. Focal therapy for prostate cancer: The current status. Prostate Int. 2015;3:35-41.

Mendez MH, Joh DY, Gupta R, Plascik TJ. Current trends and new frontiers in focal therapy for localized prostate cancer. Curr Urol Rep. 2015;16:35.

Miano R, Asimakopoulos AD, Da Silva RD, Bove P, Jones SJ, De La Rosette JJ, Kim FJ. Focal therapy for prostate cancer: Current status and future perspectives. Minverva Urol Nefrol. 2015;67:263-280.

Patel P, Young JG, Mautner V, Ashdown D, Bonney S, Pineda RG, et al. A Phase I/II clinical trial in localized prostate cancer of an adenovirus expressing nitroreductase with CB1984. Molecular Ther. 2900 July;17(7): 1292-1299.

Pisters LL, Pettaway CA, Troncoso T, McDonnell TJ, Stephens LC, Wood CG, et al. Evidence that transfer of functional p53 protein results in increased apoptosis in prostate cancer. Clin Cancer Res. 2004;10:2587-2593.

Sanfilippo NJ, Taneja SS, Chachoua A, Lepor H, Formenti SC, et al. Phase I/II study of biweekly paclitaxel and radiation in androgen-ablated locally advanced prostate cancer. J Clin Oncol. 008;26(18):2973-2978.

Sewak S, Kosmider S, Ganju V, Woollett A, Yeow EG, Le B, et al. Phase II study of paclitaxel and vinorelbine (Pacl-Vin) in hormone-refractory metastatic prostate cancer: Double-tubulin targeting. Internal Med J. 2010;40:201-208.

Shalev M, Kadmon D, The BS, Butler EB, Aguilar-Cordova E, Thompson TC, et al. Suicide gene therapy toxicity after multiple and repeat injections in patients with localized prostate cancer. J Urol. 2000 June;163:1747-1750.

Shepard DR, Dreicer R, Garcia J, Elson P, Magi-Galluzzi C, Raghavan D, et al. Phase II trial of neoadjuvant nab-paclitaxel in high risk patients with prostate cancer undergoing radical prostatectomy. J Urol. 2009;181:1672-1677.

Shikanov S, Shikanov A, Gofrit O, Nyska A, Corn B, Domb AJ. Intratumoral delivery of paclitaxel for treatment of orthotopic prostate cancer. J Pharmaceutic Sci. 2009;98(3):1005-1014.

Sonpavde G, Thomson TC, Jain RK, Ayala GE, Kurosaka S, Edamura K. GLIPR1 tumor suppressor gene expressed by adenoviral vector as neoadjuvant intraprostatic injection for localized intermediate or high-risk prostate cancer preceding radical prostatectomy. Clin Cancer Res. 2011 Nov 15;17(2):1-19.

Trudel S, Trachtenberg J, Toi A, Sweet J, Li ZH, Jewett M, et al. A Phase I trial of adenovector-mediated delivery of interleukin-2 (AdIL-2) in high-risk localized prostate cancer. Cancer Gene Therapy. 2003;10:755-763.

van der Linden RRM, Haagmans BL, Mongiat-Artus P, van Doornum GJ, Kraaij R, Kadmon D, et al. Virus specific immune responses after human neoadjuvant adenovirus-mediated suicide gene therapy for prostate cancer. European Urol. 2005;48:153-161.

van Soest RJ & de Wit R. Irrefutable evidence for the use of docetaxel in newly diagnosed metastatic prostate cancer: Results from the STAMPEDED and CHAARTED trials. BMC Medicine. 2015;13:304.

Urakami S, Igawa M, Kikuno N, Yoshino T, Kishi H, Shigeno K, et al. Combination chemotherapy with paclitaxel, estramustine and carboplatin for hormone refractory prostate cancer. J Urol. 2002;168:2444-2450.

Williamson SK, Johnson GA, Maulhardt HA, Moore KM, McMeekin DS, Schulz TK, et al.. A phase I study of intraperitoneal nanoparticulate paclitaxel (Nanotax®) in patients with peritoneal malignancies. Cancer Chemother Pharmacol. 2015 May;75(5):1075-1087.

APPENDIX A: ECOG PERFORMANCE SCALE

Patient performance status will be graded according to the Eastern Cooperative Oncology Group (ECOG) scale* as described below.

Grade	ECOG PERFORMANCE STATUS DESCRIPTION
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

APPENDIX B: SEXUAL HEALTH INVENTORY FOR MEN (SHIM)

PATIENT INSTRUCTIONS: Sexual health is an important part of an individual's overall physical and emotional well-being. Erectile dysfunction, also known as impotence, is one type of very common medical condition affecting sexual health. Fortunately, there are many different treatment options for erectile dysfunction. This questionnaire is designed to help you and your doctor identify if you may be experiencing erectile dysfunction. If you are, you may choose to discuss treatment options with your doctor.

Each question has several possible responses. Circle the number of the response that **best describes** your own situation. Please be sure that you select one and **only one response for each question**.

OVER THE PAST 6 MONTHS:

1. How do you rate your confidence that you could get and keep an erection?

Very low	Low	Moderate	High	Very high
1	2	3	4	5

2. When you had erections with sexual stimulation, how often were your erections hard enough for penetrat (entering your partner)?

No sexual activity	Almost never or none	A few times (much less than half the time)	Sometimes (about half the time)	Most times (much more than half the time)	Almost always or always
0	1	2	3	4	5

3. During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?

Did not attempt intercourse	Almost never or none	A few times (much less than half the time)	Sometimes (about half the time)	Most times (much more than half the time)	Almost always or always
0	1	2	3	4	5

4. During sexual intercourse how difficult was it to maintain your erection to completion of intercourse?

Did not attempt intercourse	Extremely difficult	Very difficult	Difficult	Slightly difficult	Not difficult
0	1	2	3	4	5

5. When you attempted sexual intercourse, how often was it satisfactory for you?

Did not attempt intercourse	Almost never or never	A few times (much less than half the time)	Sometimes (about half the time)	Most times (much more than half the time)	Almost always or always
0	1	2	3	4	5

SCORE: _____

Add the numbers corresponding to questions 1 - 5. If your score is 21 or less, you may want to speak to your doctor.

APPENDIX C: INTERNATIONAL PROSTATE SYMPTOM SCORE (I-PSS)

In the past month:	Not at All	Less than 1 in 5 Times	Less than Half the Time	About Half the Time	More than Half the Time	Almost Always	Your score
1. Incomplete Emptying How often have you had the sensation of not emptying your bladder?	0	1	2	3	4	5	
2. Frequency How often have you had to urinate less than every two hours?	0	1	2	3	4	5	
3. Intermittency How often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
4. Urgency How often have you found it difficult to postpone urination?	0	1	2	3	4	5	
5. Weak Stream How often have you had a weak urinary stream?	0	1	2	3	4	5	
6. Straining How often have you had to strain to start urination?	0	1	2	3	4	5	
	None	**1 Time**	**2 Times**	**3 Times**	**4 Times**	**5 Times**	
7. Nocturia How many times did you typically get up at night to urinate?	0	1	2	3	4	5	
Total I-PSS Score							

Score: 1-7: *Mild* 8-19: *Moderate* 20-35: *Severe*

Quality of Life Due to Urinary Symptoms	Delighted	Pleased	Mostly Satisfied	Mixed	Mostly Dissatisfied	Unhappy	Terrible
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**

APPENDIX D: NATIONAL INSTITUTES OF HEALTH PROSTATITIS SYMPTOMS QUESTIONNAIRE

Name: _____ **Date:** _____

Date of Birth: _____ **Case No:** _____

1. In the last week, have you experienced any pain or discomfort in the following areas?

a. Area between rectum and testicles (perineum)	2 - yes	1 - no
b. Testicles	2 - yes	1 - no
c. Tip of the penis (not related to urination)	2 - yes	1 - no
d. Below your waist, in your bladder or pubic area	2 - yes	1 - no

2. In the last week, have you experienced:

a. Pain or burning during urination	2 - yes	1 - no
b. Pain or discomfort during or after sexual climax (ejaculation)	2 - yes	1 - no

3. How often have you had pain or discomfort in any of these areas over the last week?

a. Never	1
b. Rarely	2
c. Sometimes	3
d. Often	4
e. Usually	5
f. Always	6

4. Which number best describes your <u>AVERAGE</u> pain or discomfort on the days that you had it, over the last week?

1	2	3	4	5	6	7	8	9	10
No Pain									Pain As Bad as You Can Imagine

Urination

5. How often have you had a sensation of not emptying your bladder completely after you finish urinating, over the last week?

a. Not at all	0
b. Less than 1 times in 5.	1
c. Less than half the time.	2
d. About half the time.	3
e. More than half the time.	4
f. Almost always.	5

6. How often have you had to urinate again less than two hours after you finished urinating, over the last week?

a. Not at all	0
b. Less than 1 times in 5.	1
c. Less than half the time.	2
d. About half the time.	3
e. More than half the time.	4
f. Almost always.	5

Impact of Symptoms

7. How much have your symptoms kept you from doing things you would usually do, over the last week?

a. None	0
b. Only a little	1
c. Some	2
d. A lot	3

8. How much did you think about your symptoms, over the last week?

a. None	0
b. Only a little	1
c. Some	2
d. A lot	3

Quality of Life

9. If you were to spend the rest of your life with your symptoms just the way they have been during the last week, how would you feel about that?

a. Delighted	0
b. Pleased	1
c. Mostly satisfied	2
d. Mixed (about equally satisfied and unsatisfied)	3
e. Unhappy	4
f. Terrible	5

Scoring the NIH-Chronic Prostatitis Symptom

Pain: Total of items 1a, 1b, 1c, 1d, 2a, 2b, 3 and 4 = ____

Urinary Symptoms: Total of items 5 and 6 = ____

Quality of Life Impact: Total of items 7, 8 and 9 = ____

The National Institute of Health Chronic Prostatitis Symptom Index (NIH-CPSI) captures the three most important domains of the prostatitis experience: pain (location, frequency, and severity), voiding (irritative and obstructive symptoms), and quality of life (including impact). This index is useful in research studies and clinical practice. (From Litwin MS, McNaughton-Collins M, Flowler FJ, et al: The NIH Chronic Prostatitis Index [NIH-CPSI]: Development and validation of a new outcome measure. J Urol 1999; 162:369-375.)