Phase 2 Trial of NanoPac® Focal Therapy for Prostate Cancer in Subjects Undergoing Radical Prostatectomy

Protocol Identifying Number: NANOPAC-2019-01

IND Sponsor: NanOlogy, LLC

Version Number: 5.0

Date: 8 Sep 2020

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALCOA-C	Attributable, Legible, Contemporaneous, Original and Accurate - Complete
ALT	Alanine Aminotransferase
ALP	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
EMR	Electronic Medical Record
EUS-FNI	Endoscopic Ultrasound Guided Fine Needle Injection
FCM	Flow Cytometry
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
IHC	Immunohistochemistry
IND	Investigational New Drug Application
IP	Intraperitoneal
I-PSS	International Prostate Symptom Score Questionnaire
IRB	Institutional Review Board
IRE	Irreversible Electroporation
ITU	Intratumoral
IV	Intravenous
LDH	Lactate Dehydrogenase
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mpMRI	Multiparametric MRI
MRI	Magnetic Resonance Imaging
TRUS	Transrectal Ultrasound Fusion

MR-TRUS	Magnetic Resonance Imaging-Transrectal Ultrasound Fusion
NCI	National Cancer Institute
NDA	New Drug Application (Marketing Application)
NIH-CPSI	National Institute of Health Chronic Prostatitis Symptom Index
NOAEL	No Observed Adverse Events Level
PCA	Precipitation with Compressed Antisolvents
PD	Pharmacodynamic
pH	Hydrogen Ion Concentration
PI	Principal Investigator
PI-RADS	Prostate Imaging–Reporting and Data System
PK	Pharmacokinetics
PRO	Patient Reported Outcome
PSA	Prostate-Specific Antigen
PSMA-PET	Prostate Specific Membrane Antigen (PSMA) Positron Emission Tomography (PET) Imaging
PT	Prothrombin Time
PTT	Activated Partial Thromboplastin Time
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDLC	Systems Development Life Cycle
SHIM	Sexual Health Inventory for Men Questionnaire
SOC	System Organ Class
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 2 Trial of NanoPac® Focal Therapy for Prostate Cancer in Subjects Undergoing Radical Prostatectomy
Protocol Number:	NANOPAC-2019-01
Version Number:	5.0
Date:	8 Sep 2020
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.

Gere diZerega
Gere diZerega (Sep 9, 2020 13:20 PDT)
_____
Signature of Sponsor's Representative

Sep 9, 2020
_____
Date

STATEMENT OF COMPLIANCE

I have read the attached protocol number NANOPAC-2019-01 entitled, *Phase 2 Trial of NanoPac® Focal Therapy for Prostate Cancer in Subjects Undergoing Radical Prostatectomy*, Version 5.0 dated 8 Sep 2020 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.

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Signature of Principal Investigator Date

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Printed Name of Principal Investigator

PROTOCOL SUMMARY

Title: Phase 2 Trial of NanoPac® Focal Therapy for Prostate Cancer in Subjects Undergoing Radical Prostatectomy

Précis: In this open-label, Phase 2 trial, 18 subjects with adenocarcinoma of the prostate will have NanoPac injected under image guidance directly into the dominant lesion in the prostate on up to three occasions, with at least 28 days between each dose, with a final study visit occurring 12 weeks, (Day 85), after the first injection. NanoPac will be injected at 15 mg/mL concentration, and the total volume administered will be up to the volume of the dominant lesion (but not to exceed 10% of the total prostate volume); all study agent will be injected directly into the dominant lesion. The volume and dose of NanoPac administered at the second and/or third injection will be no greater than the first injection. If there are any safety concerns, as assessed via individual and cumulative assessments conducted at routine intervals by the Medical Monitor, the dose concentration and/or volume may be reduced within and/or for future subjects.

Tumor volume and serum prostate-specific antigen (PSA) will be determined prior to the first NanoPac injection.

During the study the following will be collected/assessed:

- Plasma samples for pharmacokinetic (PK) analysis,
- Ejaculate for paclitaxel presence;
- Blood for routine safety, PSA levels, and flow cytometric (FCM) analysis at intervals;
- Imaging with multiparametric MRI (mpMRI) [Screening and End of Study visits];
- Imaging of the pelvic lymph nodes may be performed via PSMA-PET scan [Screening and End of Study visits]; this is an optional study assessment.
- Prostatectomy (previously scheduled) will occur approximately 92 days post first injection:
    - If a prostatectomy is not performed a biopsy will be obtained for clinical re-staging of the disease;
    - At the time of prostatectomy or biopsy, a tissue sample will be obtained for evaluation of immune response via immunohistochemistry;
    - Samples of the dominant lesion, ipsilateral lobe of the prostate, contralateral lobe of the prostate, and lymph nodes will be collected during prostatectomy

Objectives: Primary objectives:

- To determine the safety and tolerability of up to three intratumoral (ITU) injections, with at least 4 weeks between each injection; and
- To determine tumor response to up to three ITU injections of NanoPac, with at least 4 weeks between each injection.

Secondary objectives:

- To evaluate the effects on the tumor of up to three ITU injections of NanoPac, with at least 4 weeks between each injection;
- To describe the PK of up to three ITU injections of NanoPac, with at least 4 weeks between each injection.

Endpoints: The primary endpoints will be:

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

- Tumor response as demonstrated by:
    - Gleason score of dominant lesion measured at prostatectomy/biopsy compared to Screening (baseline – obtained prior to Day 1) as determined from tissue/biopsy samples;
    - Change in proportion of dominant lesion designated as adenocarcinoma at prostatectomy/biopsy compared to Screening (baseline – obtained prior to Day 1) as determined from tissue/biopsy samples.
    - Proportion of subjects with local invasion at the Final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1) as measured by mpMRI.

Secondary endpoints:

a) Effects on the tumor:
    - Change in volume of dominant lesion measured at final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1) as measured by mpMRI.
    - Change in PSA Density measured at final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1)
    - Change in PI-RADS score measured at final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1)
b) Concentration of paclitaxel in the systemic circulation post-injection;
c) Presence or absence of paclitaxel in ejaculate.
d) Presence or absence of paclitaxel in the dominant (injected) tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy (as applicable).

Exploratory endpoints:

a) Changes in Patient Reported Outcomes (PROs) between baseline and final study visit, as measured by the Sexual Health Inventory for men (SHIM), the International Prostate Symptom Score (I-PSS), and the National Institute of Health Chronic Prostatitis Symptom Index (NIH-CPSI);
b) Flow cytometric (FCM) evaluation of immune markers in blood samples obtained following NanoPac injection:
c) Determination of immune response in the prostate lesion due to NanoPac injection by immunohistochemical (IHC) staining and evaluation of the end of study tissue/biopsy samples;

Population:	Eighteen (18) men with prostate cancer
Phase:	Phase 2
Number of Sites Enrolling Participants:	Up to five (5)
Description of Study Agent:	NanoPac® (sterile nanoparticulate paclitaxel) Powder for Suspension (“NanoPac”) for direct injection into the dominant lesion at a concentration of 15 mg/mL in an injection volume of up to the volume of the dominant lesion (not to exceed 10% of total prostate volume).

Study Duration:	The study duration will be up to 18 months.
Participant Duration:	The study duration is estimated to be approximately 14 weeks for each subject. Additional follow-up may occur.

1 KEY ROLES

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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 pharmacology studies of NanoPac in the PC-3 human prostate carcinoma nude mouse tumor xenograft model, Study PC3-e317 and Study PC3-e319. Results for all NanoPac-treated animals were indicative of potential therapeutic activity. Additional data is presented in the NanoPac Investigator's Brochure.

In Study Number P-PPr-01-2015, 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. Treatments with NanoPac and vehicle were by intratumoral (ITU) injection. Treatment with either a single dose or three once-weekly doses of ITU 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 Number PD-PPr-02-2016, the efficacy of NanoPac was evaluated as part of a NanoDoce (sterile nanoparticulate docetaxel) trial in female NCI Ath/nu mice. NanoPac was administered ITU injection 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.

In a GLP toxicity and toxicokinetic (TK) study (Study Number P-TR-06-2018) evaluating a single injection of NanoPac into the prostate of rats at 4 mg/mL (1.9-2.2 mg/kg), 10 mg/mL (4.7-5.5 mg/kg), or 40 mg/mL (18.2-21.6 mg/kg) at a fixed volume of 0.18 mL, at the terminal interval on Day 4, findings of necrosis with mixed inflammation and fibrin deposition were noted in the prostate, with mineralization (kidneys), and mixed inflammation and fibrin deposition (urinary bladder, seminal vesicles and iliac lymph nodes) noted in the adjacent tissues. At the recovery

interval on Day 28 (± 1), similar findings were observed, with partial/ongoing to complete resolution. Based on the study findings, 18.2-21.6 mg/kg (40 mg/mL) was considered the NOAEL, with an associated $AUC_{0\text{-}672}$ of 517 ng·hr/mL.

Clinical Summary:

Two clinical studies with NanoPac have been completed. Additional data from these clinical trials is presented in the NanoPac Investigator's Brochure. Ongoing clinical trials using NanoPac include direct injection to pancreatic tumors via endoscopic ultrasound guided fine needle injection (EUS-FNI) (NCT03077685) and injection of NanoPac into mucinous cystic neoplasms of the pancreas by EUS-FNI (NCT03188991).

Phase 2a Study in Prostate Cancer

A Phase 2a study of NanoPac focal therapy of prostate cancer, NANOPAC-2016-02 (NCT03077659), was completed. Subjects received intraprostatic injection of NanoPac at 6, 10, and 15 mg/mL in an injection volume of 20% of the lobe of the prostate containing the dominant lesion, and were followed for 4 weeks for safety and tolerability prior to prostatectomy.

The primary objective of the study was to evaluate the safety and tolerability of NanoPac when injected into the lobe of the prostate containing the dominant lesion. Sixteen subjects were enrolled and received study agent according to the protocol. The majority of subjects experienced treatment emergent adverse events (TEAEs) as expected, but with minimal frequency, severity, and relation to NanoPac. Overall, the most frequent TEAEs occurred in the system organ class (SOC) of gastrointestinal disorders, most of which were in the 6 mg/mL cohort. There was no obvious dose-response relationship with respect to the frequencies of the TEAEs at the three concentrations of NanoPac. Compared to Taxol, no dose-limiting toxicities (DLTs) or toxicities typically attributable to paclitaxel such as neutropenia, thrombocytopenia, peripheral neuropathy, and hypersensitivity reactions such as angioedema and urticaria were reported. There were no serious adverse events (SAEs), deaths, or discontinuations from the study due to adverse events (AEs).

Pharmacokinetic (PK)/pharmacodynamic (PD) modeling of Taxol-induced neutropenia suggests toxicity is related to the duration and extent of systemic exposure to paclitaxel which correlates with a threshold plasma paclitaxel concentration of ≥ 42.7 ng/mL; in contrast, plasma paclitaxel concentrations observed with NanoPac had C_{max} values of 19 to 20 ng/mL recorded at the earliest sample viz. the 1-hour timepoint. By comparison, a standard intravenous (IV) dose of paclitaxel of 175mg/m^2 BSA administered over 3 hours resulted in a C_{max} of 3,650 ng/ml (Paclitaxel Drug Label Information – Teva), about 192x higher than the mean NanoPac concentration achieved in the 15mg/mL cohort 1 hour after injection. In alignment with this, from a toxicity and safety perspective, intraprostatic injection of NanoPac appears to have a better risk profile compared to IV Taxol administration. The original hypothesis of NANOPAC-2016-02, that direct injection of NanoPac into the prostate would result in limited, if any, systemic exposure to paclitaxel and should therefore result in only low-grade and transitory AEs is therefore supported by the safety findings in the study.

The secondary objectives of the study were to evaluate 1) tumor response as determined by change in image volume on multiparametric MRI (mpMRI) and histologic evaluation via biopsy; 2) the 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; 3) the concentration of paclitaxel in the systemic circulation post-injection and prior to prostatectomy; 4) 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; and 5) assess the effects of NanoPac on sexual health, urination, and symptoms of chronic prostatitis as measured

by the Patient Reported Outcomes (PROs) Sexual Health Inventory for men (SHIM), International Prostate Symptom Score (I-PSS), and National Institute of Health Chronic Prostatitis Symptom Index (NIH-CPSI).

Due to the small size of the study and the natural slow rate of progression of prostate cancer, the majority of the individual outcomes showed no changes or variable results across the three concentrations of NanoPac over 4 weeks of exposure to NanoPac. The mean volume of the prostate increased between the time of injection and prostatectomy, likely due to the local inflammatory reaction generated by NanoPac. Reflecting this, the sexual health as measured by the SHIM deteriorated over the course of participation, which can be explained by the increased local pressure effects created by the increased prostate volume.

Notably, the mean total Gleason score remained stable in the 6 mg/mL and 10 mg/mL cohorts, and improved in the 15 mg/mL cohort, implying a possible dose-response relationship. The proportion of the primary lesion considered adenocarcinoma improved in the 6 mg/mL and 15 mg/mL cohorts, but increased in the 10 mg/mL cohort. When defining efficacy as stable or improved Gleason score, stable or improved proportion of tissue considered adenocarcinoma, or stable or improved evidence of local invasion, 1) 13 of 16 subjects had stable or improved Gleason scores (all in the 15 mg/mL cohort); 2) 11 of 16 subjects had stable or improved proportions of cancer lesion tissue defined as adenocarcinoma (6 of 11 subjects showed reductions in proportion of tissue identified as adenocarcinoma while 5 showed no change); and 3) 11 of 16 subjects had stable or improved levels of invasion by the cancerous tissue (4 of the 11 subjects showed less invasion into the surrounding tissues, while 7 subjects showed stable disease).

When combining the same three parameters above, and defining an improvement in those parameters as a weighted positive response in the following manner, 1) if one parameter is improved and the other two parameters are stable, then the subject is deemed to have improved, 2) if two parameters improved and the remaining parameter remained stable or deteriorated, then the subject is deemed to have improved, or 3) if one parameter improved and one deteriorated, the subject is deemed stable and not an improvement, then 6 (60%) subjects in the 15 mg/mL cohort improved compared to baseline, whereas 1 (33.3%) subject in each of the 6 mg/mL and 10 mg/mL cohorts improved.

Overall, the primary objective of the study was achieved, i.e. preliminary evidence indicates that direct injection of NanoPac into the prostate appears safe and tolerable. The limited duration and size of the study prevents definitive evaluation of efficacy, but outcomes warrant further development of NanoPac as a focal therapy for prostate cancer.

Phase 1 Study in Peritoneal Malignancies

A Phase 1 study of intraperitoneal (IP) NanoPac in subjects with peritoneal malignancies, HSC#11140 (NCT00666991) was completed. The results of this study were published by Williamson 2015 in the journal *Cancer Chemotherapy and Pharmacology*.

HSC#11140 was a dose-escalating study evaluating 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 subjects were enrolled in the study. 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 subject. 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 12 subjects had progressive disease. Five of 21 subjects with advanced cancers survived longer than 400 days after initiation of IP NanoPac treatment.

Relevant Literature:

Several authors have reported results from human trials involving intraprostatic injection of various agents (such as viral vectors) for the treatment of local prostate cancer (DeWeese 2001; Johannsen 2007a; Johannsen 2007b; Finkelstein 2012; Trudel 2003; Pisters 2004; Gulley 2013; Kramer 2001; Sonpavde 2011; Patel 2009; Shalev 2000; Fujita 2006; Belldegrun 2001; van der Linden 2005; Freytag 2002).

Reports of IV administration of paclitaxel, often in combination with other agents and/or radiotherapy, have demonstrated efficacy for the treatment of locally advanced and metastatic prostate cancer (Sanfilippo 2008; Hudes 1997; Shepard 2009; Sewak 2010; Hussain 2012; Urakami 2002; Berry 2004; Hudes 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. 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. 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 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 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 2015).

2.2 RATIONALE

This Phase 2 study will include subjects with adenocarcinoma of the prostate scheduled to undergo prostatectomy. The study design allows for a safety evaluation of ITU injection of NanoPac into a prostate tumor as focal therapy prior to prostatectomy. NanoPac will be injected on up to three occasions 4 weeks (28 days) apart, with prostatectomy scheduled approximately 13 weeks (92 days) after the first injection. The proliferative kinetics of prostatic cancer are known to be slow and variable both within a tumor and between different Gleason grades (Werehera 2011). It is hypothesized that the period between first injection and prostatectomy is sufficient for initial indications of efficacy.

The prior clinical trial (NANOPAC-2016-02) evaluating a single intraprostatic injection of NanoPac concentrations up to 15 mg/mL showed systemic C_{max} concentrations about 0.5% of that of a standard 175mg/m^2 IV dose administered over 3 hours. We hypothesize that direct injection of NanoPac into the prostate tumor on three separate occasions 28 days apart will result in limited systemic exposure to paclitaxel and should therefore result in only low-grade and transitory AEs.

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 2013, Shikanov 2008; van Soest 2015). Direct injection, as opposed to IV administration, of paclitaxel would allow for higher concentrations of drug to target local disease with reduced systemic toxicity. Direct injection of NanoPac to the tumor within the prostate 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

In the initial clinical study of intraprostatic NanoPac (NANOPAC-2016-02), no significant side effects considered related to NanoPac were reported. 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

The initial clinical study of intraprostatic NanoPac (NANOPAC-2016-02) showed some positive indications of potential therapeutic activity. 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. Paclitaxel, the active pharmaceutical ingredient of NanoPac, is effective in the IV treatment of metastatic prostate cancer. 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 injections, and to evaluate the tumor response to NanoPac injected directly into the prostate tumor on up to three separate occasions 4 weeks (28 days) apart, in subjects diagnosed with prostate cancer and planning prostatectomy.

The secondary objectives are to assess the effects on the tumor, including changes on mpMRI parameters, PSA Density and PI-RADS after NanoPac injections; and to describe the PK of NanoPac injected directly into the tumor.

In addition, changes in PROs by means of questionnaires will be assessed, immunohistochemistry (IHC) will be evaluated on the excised tissue and lymph nodes (as available), and FCM will be performed on blood samples to ascertain changes in immunologic response.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

The proposed study is a Phase 2, open-label trial in 18 subjects with localized prostate cancer scheduled for prostatectomy. Subjects will have NanoPac injected under image-guided transrectal ultrasound (e.g. magnetic resonance imaging-transrectal ultrasound fusion [MR-TRUS]) or via transperineal injection with ultrasound, directly into the dominant lesion of the prostate cancer on up to three separate occasions 4 weeks (28 days) apart. NanoPac will be administered at a concentration of 15 mg/mL to all subjects, and the total volume available for administration will be the same volume as the volume of the lesion as determined by mpMRI prior to first injection, but not to exceed 10% of the total prostate volume. The same volume and dose of NanoPac will be available for administration at the second and third injection. Subjects will be scheduled to undergo radical prostatectomy approximately 92 days (13 weeks) after the first injection of NanoPac.

Prior to study entry, subjects will have undergone 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 volume of the prostate and the volume of the lesion to be treated will be calculated based on an mpMRI scan conducted prior to study participation; if not available, an mpMRI will be conducted to obtain details prior to first injection.

The patient population includes subjects with localized cancer scheduled for prostatectomy who are classified as <T3 per TNM classification, Gleason score ≥ 6. The prostate tumor must be able to be visualized on mpMRI.

Prostate tissue and biopsies will be obtained at the time of prostatectomy (Day 92). If prostatectomy is delayed for any reason, a biopsy will still be performed for tumor restaging. If prostatectomy is not significantly delayed, the biopsy can be taken when the delayed prostatectomy occurs. If the prostatectomy has been cancelled, the biopsy should be taken as planned. The biopsy location will aim to be same location as the pre-study biopsy for comparison purposes. Routine processing for histopathology will be performed at the local institution and a portion of the biopsy material will be provided on slides to a central laboratory for IHC staining and reporting.

The PK of NanoPac to determine the systemic exposure to paclitaxel will be evaluated at each visit from the day of first injection. Ejaculate samples provided during the study will also be evaluated for the presence or absence of paclitaxel.

Blood samples for FCM evaluation will be obtained prior to the first NanoPac injection on Day 1, at the visit on Days 15, 43, and 71, and again at the final clinic visit on Day 85 to assess for any immune response detectable in the blood. If Day 43 is an injection visit for a subject, the sample is to be taken prior to NanoPac injection.

The presence or absence of paclitaxel in the dominant (injected) lesion, ipsilateral lobe, contralateral lobe, and regional lymph nodes will be evaluated from the prostatectomy samples (as applicable).

4.1.1 PRIMARY OBJECTIVES AND ENDPOINTS

1. Safety and tolerability as demonstrated by:
    a. AEs, changes in laboratory assessments, physical examination findings, and vital signs.

2. Tumor response as demonstrated by:
    a. Gleason score of dominant lesion measured at prostatectomy/biopsy compared to Screening (baseline – obtained prior to Day 1) as determined from tissue/biopsy samples.
    b. Change in proportion of dominant lesion designated as adenocarcinoma at prostatectomy/biopsy compared to Screening (baseline – obtained prior to Day 1) as determined from tissue/biopsy samples.
    c. Proportion of subjects with local invasion at the Final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1) as measured by mpMRI.

4.1.2 SECONDARY OBJECTIVES AND ENDPOINTS

The secondary endpoints will be:

1. Further assessment of effects on the tumor, as demonstrated by:
    a. Change in volume of dominant lesion measured at final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1) as measured by mpMRI.
    b. Change in PSA Density measured at final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1)
    c. Change in PI-RADS score measured at final study visit (Day 85) compared to Screening (baseline – obtained prior to Day 1)
2. PK evaluation:
    a. Concentration of paclitaxel in the systemic circulation post-injection;
    b. Presence or absence of paclitaxel in the ejaculate at Days 15, 43, 57, end of study Day 85; and
    c. Presence or absence of paclitaxel in the dominant (injected) tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes excised during prostatectomy (as applicable).

4.1.3 EXPLORATORY ENDPOINTS

1. Changes in PROs between baseline and end of study Day 85, as measured by the SHIM, the I-PSS, and the NIH-CPSI;
2. FCM evaluation of immune response in blood samples following NanoPac injection(s);
3. Determination of immune response in the prostate lesion due to NanoPac injection by IHC staining and evaluation of the end of study biopsy;

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

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

- At least 18 years of age;
- Histopathologically proven adenocarcinoma of the prostate:
    - Localized cancer;
    - Subjects with tumors classified as <T3 per TNM classification, Gleason score ≥ 6;
- Prostate tumor must be able to be visualized on mpMRI;
- Already considered to be candidate for radical prostatectomy
- Considered 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
    - Calculated Creatinine Clearance ≥ 30 ml/min
    - Normal PT/INR and PTT;
- ECOG of 0 or 1;
- International Prostate Symptom Score (I-PSS) ≤ 20;
- If sexually active, willing to use double condoms from time of NanoPac injection until prostatectomy;
- Agree to all study procedures and provide signed informed consent;

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, including surgery for any benign condition (such as TURP);
- 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) while on study 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 (e.g. radiation, HIFU, cryotherapy, Focal Irreversible Electroporation, Photodynamic Therapy, Laser Induced Thermometry);
- Any other condition (e.g., psychiatric disorder) that, in the opinion of the Investigator, may interfere with the subject'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 18 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 confirmed suitable for Screening (have confirmed prostate cancer prior to consenting to the study), screened for eligibility, and will proceed to treatment in accordance with the protocol.

Accrual of subjects is expected to 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, and therefore in all study documentation this subject would be considered a screenfail subject. Reasons for failing the screening will be documented.
- 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 AEs, 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) (see Section 7.3.8 Early Termination Visit).

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 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 terminated 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 the other party. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform the Institutional Review Board (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 safety concern requiring discussion with the Medical Director and with the PI may result in termination of study based on unacceptable risk, which will consider all safety evaluations and DLTs.
- Insufficient compliance with protocol requirements;
- Data that are not sufficiently complete and/or evaluable.

In the event the study is temporarily suspended, the 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 Product Storage and Stability).

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 IFU insert will be provided to the Pharmacy ahead of the first subject being enrolled. The IFU will contain information on the reconstitution of the drug for 15mg/mL dose level, 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, the volume contained in the syringe, and visit information as well as date and time of preparation, 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, volume of NanoPac required and the volume of the prostate lesion. 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, or as needed per site process. Sites may have electronic systems (such as Vestigo) set up to provide and manage all of this documentation.

Once the drug has been reconstituted to the required 15 mg/mL, the volume for potential use (being the volume of the prostate 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, NanoPac will be administered as an ITU injection into the dominant lesion of prostate cancer.

Administration will occur on up to three occasions: Day 1, 4 weeks after that at Day 29, and again 4 weeks later at Day 57. If the subject is not able to have the second injection at Day 29 they may receive the second injection at Day 43; however, they will not be eligible to receive a third injection if the second injection occurs at this delayed time point.

An enema may be performed 12 hours prior to each injection procedure to optimize ultrasound visualization.

The subject 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 200 mL, the injection procedure will be undertaken. If the volume is less than 200 mL, the subject will be encouraged to drink water to increase the bladder volume to 200 mL. A volume of 200 mL is chosen to dilute any drug that can potentially extravasate into the bladder at the time of the prostate injection. The subject can have the option of a catheter being passed into the bladder to fill to the desired volume.

The subject 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 22G x 10" needle connected to the syringe containing the study agent will be inserted transrectally into the dominant lesion of the prostate under real-time TRUS guidance or transperineally via ultrasound. The needle will be retained in the rectum or perineum and reinserted into the prostate lesion several times in a fanning pattern to infuse the dominant lesion of the prostate with NanoPac.

After the procedure, the subject will be asked 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.

6.1.6 ROUTE OF ADMINISTRATION

NanoPac will be administered as an ITU injection to the tumor within the prostate under TRUS guidance.

6.1.7 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

No dose adjustment or modification in an individual subject is anticipated. The administered volume used for the first injection will also be available/used for the second and third injections, keeping dosing consistent.

However, the Medical Monitor will assess the safety parameters for each subject prior to second and/or third injections in order to provide additional safety information to assist with the Investigator's decision to proceed to the next injection. Documentation of this review will be provided for the study files, and provided to the treating Investigator ahead of the scheduled injection visit. If during this safety monitoring it is determined that it would be more appropriate to lower the volume delivered for the second or third procedure, dose reduction may occur. In this situation, the Medical Monitor will discuss with the Investigator to determine appropriate action required, and the reasoning resulting in this reduction will be documented for the study files and in the EDC.

6.1.8 DURATION OF THERAPY

NanoPac is being injected directly into the prostate lesion on up to three occasions 4 weeks (28 days) apart. The subjects will be followed for 85 days from the day of first injection and will attend regular clinic follow-up visits during this time; prostatectomy is scheduled to occur within a week of the final clinic visit, but will allow for up to 6 weeks following last NanoPac injection. The duration of therapy is therefore 13 weeks, or may be up to 15 weeks if the subject receives all three injections.

6.1.9 TRACKING OF DOSE

Subjects will receive 15 mg/mL ITU NanoPac at a volume of up to the same volume as their prostate lesion, but no more than 10% the volume of the total prostate. The first injection will determine the maximum possible volume that can be administered safely, in the opinion of the Investigator as determined during that first injection

procedure. The volume administered at the first injection will be the same amount provided for injection at the second and third injections, as applicable. If not all the volume is able to be administered, for any reason, the actual volume administered will be recorded and the dose delivered can therefore be calculated for each injection. It is anticipated that the same volume will be able to be administered at each injection; up to the volume administered at the first injection will be provided for the second and third injections, the volume will not be higher than at the first injection. In the event there is significant resistance to the injection of NanoPac, the rate of injection of NanoPac should be reduced in an attempt to inject the full dose of NanoPac. Ultimately the Investigator will determine how much is delivered and this will be documented accordingly.

Actual volumes administered at each injection will be documented in the subject source and in the EDC.

6.1.10 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 subjects (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 first NanoPac administration, including demographics and review of previous medical records;
- Review and documentation of concomitant prescription and non-prescription medications;
- Review and documentation of diagnosis of adenocarcinoma of the prostate (including biopsy results and mpMRI results) and previous treatments including surgical and chemotherapeutic records. A copy of the pathology report confirming the diagnosis must be filed in the subject's study record.
    - The biopsy results will be used to identify the dominant lesion for injection.
    - The mpMRI results will provide prostate volume, and dimensions (x3) and volume of the lesion to be injected;
- Comprehensive physical examination, including ECOG Performance Status assessment (Appendix A), and vital signs (blood pressure, heart rate, temperature, body weight and height);

- Questionnaires for determination of PROs, namely SHIM (Appendix B), I-PSS (Appendix C), and NIH-CPSI (Appendix D), will be completed at Screening, prior to NanoPac injection (may be Day 1, Day 29, Day 43, and/or Day 57) and at the final clinic visit (Day 85);
- Sample collection and processing for clinical laboratory assessments (see Section 7.2.1 Clinical Laboratory Evaluations);
- Plasma samples for PK analysis will be taken (see Section 7.2.2 Other Assays and Procedures);
- Possible bladder scan prior to NanoPac injections to assist with visualization, and following voiding after the injection procedure if required;
- Imaging with mpMRI prior to first injection and at the final study visit (one week prior to prostatectomy [Day 85]). The reports must provide three dimensions and a volume measurement for the lesion being treated;
- PSMA-PET scans may be performed prior to first injection and at the final study visit (one week prior to prostatectomy; (Day 85) if the site has access to this scan;
- Direct injection of NanoPac into the dominant lesion under TRUS guidance at Day 1, Day 29, and Day 57 [or at Day 1 and Day 43 for those subjects not able to have a second injection at Day 29, therefore not having a third injection];
- Ejaculate will be collected (see Section 7.2.2);
- Evaluation of excised prostate and lymph nodes after prostatectomy, as applicable.

Subjects will be required to complete an electronic diary on a daily basis while enrolled in the study. Completion of up to 10 questions will be requested to occur once daily, before bedtime, to assess the previous 24-hour performance.

The site coordinator will create a subject-specific user account within the TrialMaster system and train the subject on how to complete the forms. The coordinator will also request a contact e-mail address for the subject diary account to be established; no other study personnel have access to this information. The subject receives an email, follows the link, uses the temporary system assigned password to log into the system and establishes their private password. This securely activates the account the subject will use to complete the diary. An access and user instruction leaflet will be provided to the subject. The data is saved within the protocol specific TrialMaster database.

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

Sample collection and processing for clinical laboratory assessments will be taken at Screening and at all other visits after the first injection of NanoPac. Samples on NanoPac injection days will be collected prior to the injection procedure.

Clinical laboratory parameters to be measured will be the following:

- 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, and uric acid;
- Red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), white blood cell count (WBC) including differential, reticulocyte count, and platelet count;
- Urinalysis including hydrogen ion concentration (pH), RBC, WBC, protein, and glucose;
- Serum PSA.

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

PK Samples

PK samples will be taken at 1 and 2 hours post-injection at the first injection, and pre-injection, 1 and 2 hours post-injection at second and/or third injection visits, and at all other study visits. PK samples on injection days will allow for a +/- 10-minute window around the injection sample timepoints, with T0 = start time of injection.

Biopsy

The biopsy(ies) taken immediately prior to prostatectomy or immedately prior to processing the tissue from the 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 biopsy sample(s) will be sent to local lab and processed for histologic evaluation and comparison to pre-study biopsy sample where tumor was previously identified. The lab will prepare 3 slides from both biopsy timepoints (pre-study participation and pre-prostatectomy or immediately prior to processing the tisse from the prostatectomy) for additional IHC staining at NeoGenomics Laboratories, Inc. (NeoGenomics) if sufficient tissue remains available, when requested by Sponsor for shipping.

A second biopsy sample will be prepared for paclitaxel analysis at Covance Laboratories; this sample will be from fresh tissue (not formalin fixed) and kept frozen until shipment requested by Sponsor.

Prostatectomy Tissue

Routine clinical evaluation will be performed at the local pathology laboratory on the prostatectomy sample. For study purposes, fresh samples, (prior to fixing in formalin), will also be obtained from the excised prostate and pelvic lymph nodes for determination of the presence of paclitaxel on specimens sent to Covance Laboratories; these samples will be kept frozen until Sponsor requests shipment; tissue will be processed at the clinical site for shipping to NeoGenomics where IHC staining and evaluation will be performed.

If prostatectomy is significantly delayed, a biopsy should be performed at time surgery was planned. If only slightly delayed, the biopsy and prostatectomy can be done together.

Imaging

Imaging with ultrasound and mpMRI will have been conducted prior to study participation as part of the routine evaluation and confirmation of adenocarcinoma. If an mpMRI has not been done during routine evaluation (within one month of informed consent), it will be required during the Screening period. These results will serve as the pre-treatment (or Baseline) data, prior to NanoPac injection; another study-specific mpMRI will then be conducted at the final study visit (one week prior to prostatectomy) at Day 85.

PSMA-PET

Performing PSMA-PET scans will be optional and if done, will be done prior to the first injection and at the final study visit (one week prior to prostatectomy, Day 85) to assess the presence of tumor in the lymph nodes.

Ejaculate

In the interval between NanoPac injections and prostatectomy, ejaculate will be collected at Day 15,Day 43, Day 57 and Day 85 for determination of the presence of paclitaxel. Ejaculate may be collected at home the day prior to the study visit and stored in a freezer overnight.

Immune response assessed by FCM

Blood samples will be obtained prior to the first NanoPac injection and then at visits on Day 15, Day 43, Day 71 and end of study Day 85. For bloods drawn on Day 43, if this is an injection visit the FCM bloodwork should be drawn with the pre-injection PK sample prior to NanoPac injection. Blood will be drawn, prepared, and shipped same day for overnight delivery to NeoGenomics for FCM evaluation.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

PK samples will be drawn at the specified time/visit and stored frozen on-site until the sponsor indicates shipping should be performed, at which time they will be batch-shipped to Covance Laboratories (Madison, WI) for analysis.

Following prostatectomy surgery, a representative section of the prostate tumor (from as close to the biopsy of the tumor as possible), the ipsilateral and contralateral lobes as well as up to four associated lymph nodes will be obtained from the excised tissue and will be immediately frozen and prepared for shipment to Covance Laboratories for analysis of the presence of paclitaxel in the excised sample; this will be done on fresh tissue, (prior to fixing in formalin). 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, and for preparation of slides to be sent to NeoGenomics for IHC staining and evaluation. Should there not be enough tissue available for both samples, the tissue going to Covance for paclitaxel analysis takes precedence over the IHC analysis.

Immediately prior to, or from the tissue sample collected from the prostatectomy, a biopsy sample(s) will be taken and sent to Pathology for histopathologic evaluation and comparison with the initial biopsy results obtained confirming the presence of adenocarcinoma. Slides will also be prepared and sent to NeoGenomics for IHC staining and evaluation if enough tissue sample is available.

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 and for entry to the EDC.

Samples of ejaculate obtained at the time/visit specified will be snap frozen and stored on site until shipping is confirmed to be required by the sponsor, at which time all samples will be shipped to Covance 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 ice packs which will be provided at the prior clinic visit.

Blood samples for FCM evaluation will be drawn into tubes supplied for this purpose, and shipped to NeoGenomics on the day the sample is obtained; samples must remain at ambient temperature until shipped.

NeoGenomics, the central laboratory for IHC evaluation and for FCM evaluation, will provide a manual to detail the requirements for the preparation of the slides and for the blood samples for shipping to their facility.

7.2.4 SPECIMEN SHIPMENT

Routine laboratory samples and PSA samples will be sent to the local laboratory upon collection.

Samples for paclitaxel assessment will be batch shipped, in a temperature-controlled environment, to Covance Laboratories, only when directed by the sponsor. (Ejaculate, PK and tissue from prostatectomy and biopsy will be shipped to Covance).

Tissue samples for histological evaluation will be sent to the local laboratory upon collection, and slides will be prepared at the local laboratory for shipping to NeoGenomics for IHC staining and evaluation; slides will be batch shipped when directed by the sponsor.

Blood drawn for FCM will be shipped at ambient temperature on the same day the blood is drawn, to NeoGenomics for evaluation.

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 15 weeks prior to the scheduled prostatectomy, and therefore 14 days prior to the first NanoPac injection:

- Written informed consent including comprehensive discussion of the study schedule, procedures, and subject protocol requirements;
- Complete medical history, including demographics and review of previous medical records;
- Review and documentation of adenocarcinoma of the prostate diagnosis (including biopsy results, ultrasound results, and mpMRI results obtained within one month 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 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);
- Subject completion of SHIM, I-PSS, and NIH-CPSI questionnaires (Appendices B-D);
- Sample collection and processing for clinical laboratory assessments:
    - Hematology, biochemistry, urinalysis, 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 12 hours prior to the NanoPac dosing procedure at the next study visit, if applicable;
- mpMRI will be performed prior to the first injection, and considered part of the Screening procedures; this may have been performed as part of standard of care within a month prior to informed consent, and would therefore not be repeated but data from the mpMRI captured as baseline data; PSMA PET scans may be done if available to the site and performed as per schedule of mpMRI.
- Electronic Diary – the subjects will be provided secure access to an electronic diary to be completed daily throughout the study; training will be provided at the Screening visit and as needed during the study.

7.3.2 INJECTION PROCEDURE VISITS

Day 1 – Day of First NanoPac Injection

The day of the first NanoPac injection (always considered Day 1) will be scheduled relative to prostatectomy, with the first NanoPac injection occurring 13 weeks (92 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 2 days either side 92 days prior to prostatectomy, plus or minus 2 days = Days 90-94) is permitted for the Day 1 NanoPac injection visit.

All subsequent follow-up study visits will be scheduled relative to the first NanoPac injection visit (**NOT prostatectomy)**, with a window of plus or minus 1 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 2 days window would permit the NanoPac injection to be scheduled on a Tuesday, Wednesday or Friday. Assuming the day of first NanoPac injection (Day 1) is a Tuesday, follow-up visits for the next 13 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 92.

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;
- Review of electronic diary data;
- Vital signs obtained (blood pressure, heart rate, temperature, and body weight);
- ECOG documented;
- Sample collection and processing for clinical laboratory assessments prior to injection:
    - Hematology, biochemistry, urinalysis, and PSA levels;
- Sample collection for FCM evaluation prior to injection;
- Subject completion of SHIM, I-PSS, and NIH-CPSI questionnaires (Appendices B-D);
- Preparation for dosing;
    - Subject may 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 (see Section 6.1.5);
- PK samples will be drawn (see Section 7.2.2);
- Concomitant medication will be reviewed and updated as necessary;
- AEs will be collected from the time of dosing;
- Subject will be provided antibiotics to take following the procedure, based on the rectal swab culture;
- Subject will be reminded to continue completing daily electronic diary entries.

7.3.3 FOLLOW-UP

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

Day 8 and Day 15

The subject will return to the clinic for the following evaluations:

- Vital signs will be obtained (blood pressure, heart rate, temperature, and body weight);
- PK samples will be taken;
- Review of electronic diary data;
- Sample collection and processing for clinical laboratory assessments (see Section 7.2.1):
    - Hematology, biochemistry, urinalysis, and PSA levels;
- Day 15 only - sample for FCM evaluation will be obtained
- AEs will be recorded;
- Concomitant medication will be reviewed and updated as necessary;
- An ejaculate sample will be obtained at the Day 15 visit only.

The subject will undergo any other evaluations determined necessary by the Investigator to ensure the subject's well-being. Subject will be reminded to continue completing daily electronic diary entries.

7.3.4 SECOND AND THIRD INJECTIONS

Day 29, Day 43, and/or Day 57 – as applicable

On the day of the second and/or third injections of NanoPac the following study-specific procedures will be conducted:

- Review of electronic diary data;
- Subject completion of SHIM, I-PSS, and NIH-CPSI questionnaires (Appendices B-D);
- ECOG Performance Status assessment (Appendix A), and vital signs (blood pressure, heart rate, temperature, and body weight);
- Sample collection and processing for clinical laboratory assessments (see Section 7.2.):
    - Hematology, biochemistry, urinalysis, and PSA levels;
- Sample for FCM evaluation will be obtained on Day 43 only, and if an injection is being performed the sample must be obtained prior to injection;
- An ejaculate sample will be collected on Days 43 and 57 only;
- Preparation for dosing;
    - Subject may 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 (see Section 6.1.5);
- PK Samples will be drawn (see Section 7.2.2);
- Concomitant medication will be reviewed and updated as necessary;
- AEs will be recorded;
- Subject will be provided antibiotics to take following the procedure, based on the rectal swab culture.

The subject will undergo any other evaluations determined necessary by the Investigator to ensure the subject's well-being. Subject will be reminded to continue completing daily electronic diary entries.

7.3.5 FOLLOW-UP VISITS

Follow-up visit days will depend on the days of second and third injection; on planned injection days, when an injection is not performed, that visit will be considered a follow-up visit.

Follow-up visits could be any of the following: Day 29, Day 36, Day 43, Day 50, Day 57, Day 64, Day 71

The subject will return to the clinic for the following evaluations:

- Review of electronic diary data;
- Vital signs will be obtained (blood pressure, heart rate, temperature, and body weight);
- PK samples will be taken (see Section 7.2.2);
- Sample collection and processing for clinical laboratory assessments (see Section 7.2.1):
    - Hematology, biochemistry, urinalysis, and PSA levels;
- A sample for FCM evaluation will be taken at Days 43 and 71;
- AEs will be recorded;
- Concomitant medication will be reviewed and updated as necessary;
- An ejaculate sample will be collected for all subjects at Days 43 and 57 only.

The subject will undergo any other evaluations determined necessary by the Investigator to ensure the subject's well-being. Subject will be reminded to continue completing daily electronic diary entries.

7.3.6 FINAL STUDY VISIT – END OF STUDY VISIT

Day 85 – (one week prior to scheduled prostatectomy)

The subject will return to the clinic for the following evaluations:

- Review of electronic diary data;
- Ejaculate will be collected;
- Subject completion of SHIM, I-PSS, and NIH-CPSI questionnaires (Appendices B-D);
- Comprehensive physical examination, including ECOG Performance Status assessment (Appendix A), and vital signs (blood pressure, heart rate, temperature, and body weight);
- Sample collection and processing for clinical laboratory assessments (see Section 7.2.1):
    - Hematology, biochemistry, urinalysis, and PSA levels
- A sample for PK analysis will be taken (see Section 7.2.2);
- A sample for FCM evaluation will be taken;
- Concomitant medications will be reviewed and updated as necessary, including post-surgical medications;
- AEs will be recorded;
- mpMRI will be performed;
- PSMA PET scan may be performed as an optional study assessment, if available to the site.

The subject will undergo any other evaluations determined necessary by the Investigator to ensure the subject's well-being. This visit is considered the final study visit for the subject. At this time the subject will exit the study with no further study follow-up requirements, unless there are ongoing AEs which require follow-up.

7.3.7 PROSTATECTOMY

Day of Prostatectomy

The subject will return for their scheduled prostatectomy. Due to the window permitted for the Day 1 NanoPac injection visit, the day of prostatectomy may occur anytime between Day 90 and Day 106.

Following surgery, samples will be provided from the excised tissue (prostate and lymph nodes) for evaluation of the presence of paclitaxel; routine histopathology and assessment of tumor cells will be performed at the local facility, and additional slides of the tumor will be provided and sent to the central laboratory (NeoGenomics) for IHC evaluations.

Following the prostatectomy, the subject will receive standard of care with their follow up continued as recommended by their treating physician.

If there has been a significant response (as seen in routine follow-up and from mpMRI) which causes the Investigator to delay prostatectomy, biopsy will be performed on the day which would have been the prostatectomy visit, to assess staging of the disease and status of subject. Biopsies will be provided for routine histopathology evaluation at the local laboratory, and slides will be provided for IHC evaluation at the central laboratory. In addition, a biopsy will be provided for determination of paclitaxel presence. If prostatectomy is delayed, the subject will be requested to provide ejaculate samples every 6 weeks until paclitaxel can no longer be detected by assay, or until prostatectomy is performed. During this time the subject must continue use of double condoms, as applicable. A further blood sample for FCM evaluations may be obtained at the time of delayed prostatectomy.

7.3.8 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, laboratory sample collection, PK sample collection, 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, AEs, and concomitant medications.

7.3.9 UNSCHEDULED VISITS

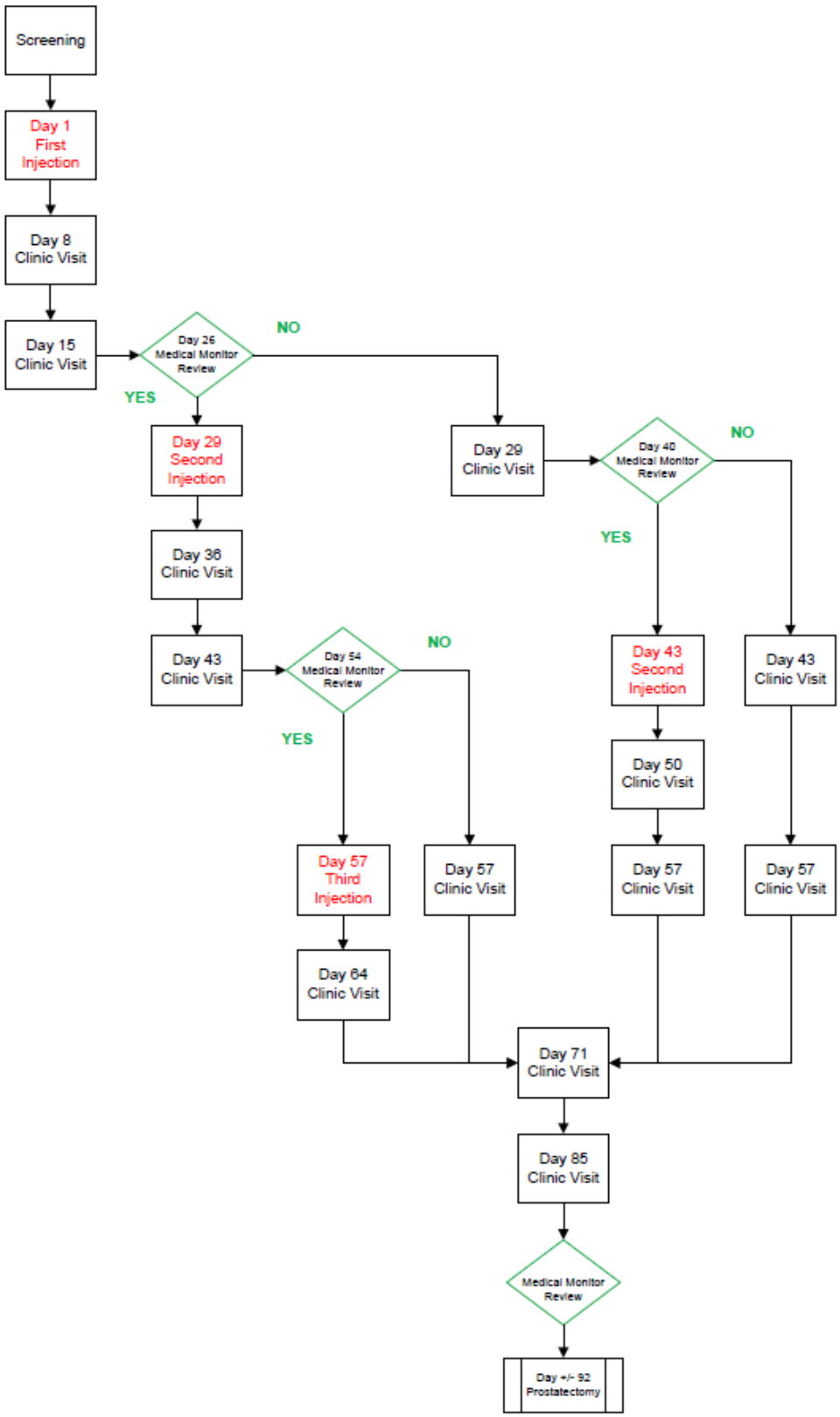
Any unscheduled visits will be documented in the source documents, and any assessments and/or evaluations performed will be noted and reviewed. If the unscheduled visit occurs after dosing but prior to surgery, the following assessments should be conducted to monitor the ongoing safety of the subject:

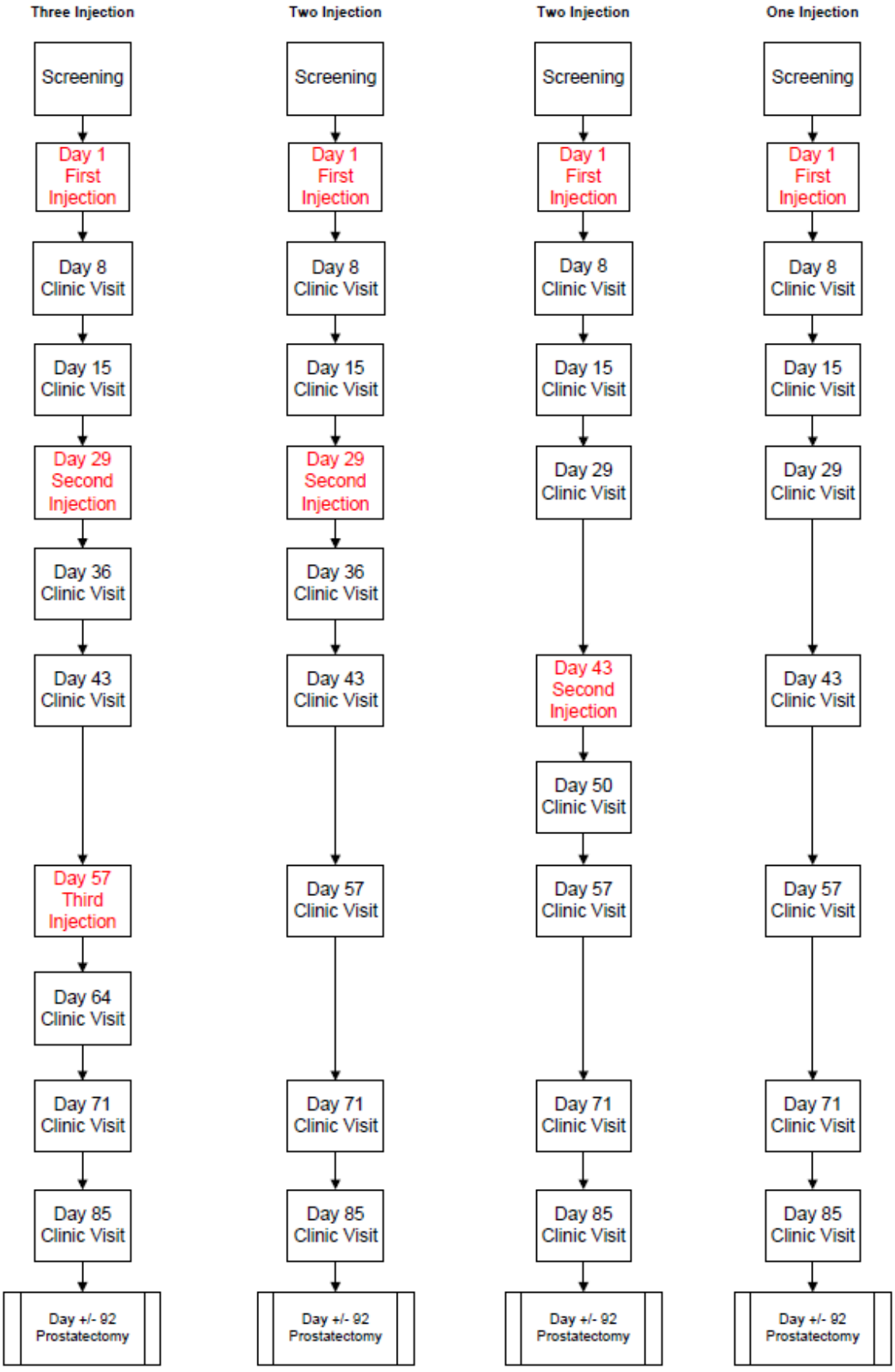
- Vital signs will be obtained;
- Review of the electronic diary data;
- AEs will be recorded;
- Concomitant medication will be reviewed and updated as necessary.

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.

7.3.10 SCHEDULE OF EVENTS TABLE

7.3.10.1 SCHEDULE OF SUBJECT VISITS




Three Injection
Screening
Day 1 First Injection
Day 8 Clinic Visit
Day 15 Clinic Visit
Day 29 Second Injection
Day 36 Clinic Visit
Day 43 Clinic Visit
Day 57 Third Injection
Day 64 Clinic Visit
Day 71 Clinic Visit
Day 85 Clinic Visit
Day +/- 92 Prostatectomy
Two Injection
Screening
Day 1 First Injection
Day 8 Clinic Visit
Day 15 Clinic Visit
Day 29 Second Injection
Day 36 Clinic Visit
Day 43 Clinic Visit
Day 57 Clinic Visit
Day 71 Clinic Visit
Day 85 Clinic Visit
Day +/- 92 Prostatectomy
Two Injection
Screening
Day 1 First Injection
Day 8 Clinic Visit
Day 15 Clinic Visit
Day 29 Clinic Visit
Day 43 Second Injection
Day 50 Clinic Visit
Day 57 Clinic Visit
Day 71 Clinic Visit
Day 85 Clinic Visit
Day +/- 92 Prostatectomy
One Injection
Screening
Day 1 First Injection
Day 8 Clinic Visit
Day 15 Clinic Visit
Day 29 Clinic Visit
Day 43 Clinic Visit
Day 57 Clinic Visit
Day 71 Clinic Visit
Day 85 Clinic Visit
Day +/- 92 Prostatectomy

7.3.10.2 SCHEDULE OF ASSESSMENTS

	Day -14	*Days 1, 29, 57 or Days 1, 43 or other*	*Days 8, 15, (29), 36, (43), 50, (57), 64, 71 as applicable*	*Day 85*	*Day 92*
	SCREENING	**DAY OF INJECTION**	**FOLLOW-UP VISITS**	**FINAL CLINIC VISIT**	**Prostatectomy / Biopsy**
Informed Consent	**X**				
Medical History [2]	**X**				
Physical Exam	**X**			**X**	
Vital Signs	**X**	**X**	**X**	**X**	
ECOG	**X**	**X**		**X**	
Daily electronic diary	**X**	**X**	**X**	**X**	
Questionnaires	**X**	**X**		**X**	
Hematology, Biochemistry, Urinalysis	**X**	**X**	**X**	**X**	
PSA	**X**	**X**	**X**	**X**	
PK Samples		**pre-inj, 1hr, 2hr [3]**	**X**	**X**	
Ejaculate collection [9]		**X**	**X**	**X**	
Rectal swab [4]	**X**				
Antibiotics		**X**			
Biopsy (Pathology and IHC)	**X[7]**				**X**
Radiologic Assessment (mpMRI)	**{ X }**			**X**	
PSMA PET Scan (optional) [10]	**X**			**X**	
NanoPac Procedure (inc all) [1]		**X**			
Blood for Flow Cytometry (FCM) [8]		**X**	**X**	**X**	
Concomitant therapy	**X**	**X**	**X**	**X**	
Adverse Events [6]	**X**	**X**	**X**	**X**	
Prostatectomy [5]					**X**
Excised tissue prep					**X**
End of Study				**X**	

[1] The day of first NanoPac injection (Day 1) will be scheduled relative to prostatectomy, with NanoPac injection occurring 92 days prior to the day planned for prostatectomy with a window of plus or minus 2 days (92 days prior to prostatectomy, plus or minus two days = 90-94 days). All subsequent study visits will be scheduled relative to the day of first NanoPac injection (NOT prostatectomy), with a window of plus or minus one day permitted.

[2] History includes all events before initiation of NanoPac treatment.

[3] PK Samples on Day 1 drawn at 1 and 2 hours post-dose; on other injection days PK samples are drawn pre-injection and at 1 and 2 hours post dose; allows for a +/- 10-minute window around samples.

[4] A rectal swab will be taken and cultured during screening to aid antibiotic prophylaxis.

[5] There is a possibility that following the end of study (Day 85) assessments, should the Investigator/physician and subject elect not to proceed to prostatectomy at that time but to delay it to a future date, the subject will be counselled regarding contraceptive use and provision of ejaculate samples every 6 weeks until paclitaxel is not detectable, or until prostatectomy is performed, whichever is first.

[6] AE determination will start immediately following initiation of study treatment.

[7] Slides from the biopsy pre-study will be provided for IHC evaluation

[8] Blood for FCM evaluation will be obtained prior to the first NanoPac injection, on Days 15, 43, and 71, and at the final study visit, Day 85

[9] Ejaculate samples will be collected on Day 15, Day 43 (prior to NanoPac injection), Day 57, and at the final study visit, Day 85.

[10] PSMA PET scan is an optional study procedure and will only be performed if available to the site.

{ X } Should be available from pre-study period; if not will need to be performed. Must be collected no earlier than one month prior to consent.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

Sponsor acknowledges that ITU injection of NanoPac into the lesion in the prostate 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). Appendix E provides a more extensive list.

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

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

Subject will be instructed to take antibiotics before/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:

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

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, the Medical Monitor will assess the safety parameters for each subject prior to second and/or third injections in order to provide the treating Investigator with any signs or symptoms that might suggest the subject is not ready for the next injection. Documentation of this review will be provided for the study files, and provided to the treating Investigator ahead of the scheduled injection visit. The ultimate decision lies with the treating Investigator.

Every 3 months the Medical Monitor will review the cumulative study data and this will be documented for the files.

8.1.1 DEFINITION OF ADVERSE EVENTS

An AE, is any untoward medical occurrence in a patient or clinical trial subject administered an investigational product, irrespective of causality. 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 AEs 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 AEs and reported on the eCRF.

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS

A SAE is any adverse event that meets at least one of following criteria:

1) Is fatal;
2) Is life-threatening, meaning the subject 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 subject 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

As this is a Phase 2 study, all unanticipated problems (UPs) will be captured as either AEs or SAEs 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 according to the NCI CTCAE v5 grades; Grades 1 through 5 have unique clinical descriptions of severity for each AE based on the general guideline as follows:

- **Grade 1 - Mild:** Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2 - Moderate:** Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
- **Grade 3 - Severe:** Or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- **Grade 4 - Life-Threatening:** Urgent intervention indicated
- **Grade 5 - Fatal:** Death related to the adverse event.

8.2.2 RELATIONSHIP TO STUDY AGENT

The following five-point scale will be used by the Investigator to rate the relationship of the AE to the study agent:

- **Definitely related:** A clinical event (including laboratory test abnormality) occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (de-challenge) should be clinically plausible. The event must be definitively associated pharmacologically, using a satisfactory re-challenge procedure, if necessary;

- **Probably related:** A clinical event (including laboratory test abnormality) with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition;

- **Possibly related:** A clinical event (including laboratory test abnormality) with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear;

- **Unlikely to be related:** A clinical event (including laboratory test abnormality) whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a

reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments);

- **Not related:** An event for which sufficient information exists to conclude that the etiology of the event is unrelated to the study agent. An alternative definitive etiology should be documented by the Investigator.

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

All AEs and SAEs must be followed until the event resolves or, in the opinion of the Investigator, become stable/or adequately managed. Subjects will be required to spontaneously report any AE. Study personnel will ask open-ended questions to obtain information about AEs at every visit. Date and time of onset and resolution (if applicable) of the AEs will be documented.

The Sponsor will report any serious, unexpected, and drug-related AEs 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 AEs (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 AEs:

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

8.4.2 SERIOUS ADVERSE EVENT REPORTING

All SAEs, including death, due to any cause which occur during this study, 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; this form may be printed and completed then scanned and sent in via email, or it

may be completed electronically and emailed directly to the Medical Monitor, and, if necessary, the SAE may be reported by phone to:

Dr. Antony Verco
Medical Monitor
US Biotest, Inc.
Email: tony.verco@usbiotest.com
Phone: 805-235-9193

24-hour Emergency Contacts:	Gere diZerega, MD Medical Director 805-630-2800	or	Antony Verco, MD Medical Monitor 805-235-9193

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 may receive up to three administrations 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), rectal bleeding, hematuria, hematospermia, and urinary retention.

AEs of special interest that occur due to any of the procedures and/or are related to the study agent (paclitaxel) 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 – Subject unable to void and requires catheterization. The decision to catheterize depends on individual subject bladder capacity and abdominal discomfort. Interim data from a TK 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 this study has been limited to not exceed 10% of the total prostate volume 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 subject 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 unlikely to occur 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 bone marrow suppression, 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. DLT information will be collected in an ongoing manner from the time of dosing until prostatectomy. DLTs considered to be SAEs (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 PI. All AEs of the below specified grades will count as DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes. DLTs include the following:

- Any death not clearly due to the underlying disease or extraneous causes;
- 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 3 non-hematologic toxicity;
- Any delay of scheduled prostatectomy due to study agent related toxicity;
- AST and ALT levels over 3x ULN;
- Symptomatic urine retention secondary to a study injection-related blockage of the prostatic urethra;
- Neutropenic fever;
- 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;
- Grade 3+ electrolyte abnormality that lasts >72 hours, unless the subject has clinical symptoms, in which case all grade 3+ electrolyte abnormality regardless of duration should count as a DLT. (Grade 3+ amylase

or lipase elevation NOT associated with symptoms or clinical manifestations of pancreatitis does not need to be counted as a DLT);

- 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 the first NanoPac injection until after prostatectomy. If a subject does not proceed to have the prostatectomy, follow-up ejaculate samples will be required to evaluate for presence of paclitaxel until it is no longer detectable or until prostatectomy is performed; until this time the subject must continue to use double-barrier birth control to prevent pregnancy in the partner.

Any pregnancy occurring in a sexual partner while a subject is in the study period between first 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 is a Phase 2 study, and will be subject to review of all safety and tolerability data in an ongoing manner. Following review, the study may be terminated.

The Medical Monitor may determine that the second injection in each/any subject may proceed as planned, however the investigator and the subject always have the right to decline to perform a second and/or third injection; the Medical Monitor may determine that it would be more appropriate to lower the second dose (by reducing the volume to be administered) to prevent any adverse findings which may appear during this period; or may determine that the safety and tolerability profiles are not acceptable and may determine that more injections are not reasonable for the safety of the subject(s); it is also possible that the Medical Monitor will advise that the study be stopped.

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 Medical Monitor and at any time may also involve the Medical Director and/or the Investigator if any concerns arise. A Safety Management Plan will be drawn up prior to study start.

All subject study data and the electronic daily diary 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, and specifically prior to a scheduled injection visit, 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 PI, as appropriate).

During the quarterly review, the Medical Monitor 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 8.4.5.

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 subject's medical records, regulatory binder, study binder, eCRFs, and source documents as needed to ensure 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 ensure compliance with the study protocol, to review and collect the subject'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. The events of special interest and/or DLTs (Sections 8.4.4 and 8.4.5) will be identified early in the trial to ensure that the Medical Monitor is provided easy to review summaries. The associated MedDRA SOC and PT terms will be approved by the Medical Monitor and included in the SAP. In addition, if requested by the Medical Monitor, medically relevant changes (e.g., 3 x the normal range) and analytes of interest will be defined in the SAP and also incorporated into the EDC edit checks once the alert ranges are established.

To enhance the focus on Safety, timeframes may be established (e.g. within 24-hours of injection) to summarize AEs and/or other events, that would become apparent with the ongoing review of the study data, to be highlighted in the Clinical Study Report (CSR).

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 2 trial, the focus will be on providing descriptive statistical summaries including tables and graphs. The clinical/medical review of these data summaries will determine possible toxicity related issues that could be associated with NanoPac and the effect, if any, of the treatments on the prostate tumor.

Missing data will not be imputed, as imputation methods are based on assumptions which could affect the interpretation of the data. With the small number of subjects and the early stage in drug development it is acknowledged that missing data is not ignorable. The missingness will be displayed and reviewed by the medical monitor as part of the overall interpretation of the trial results.

10.4.2 ANALYSIS OF THE PRIMARY ENDPOINT(S)

The primary objective of this study is to evaluate the safety and tolerability, and preliminary efficacy of NanoPac (at a concentration of 15 mg/mL) injected directly into the dominant lesion of the prostate cancer.

The primary safety assessment will be the AE data, which will also include vital signs and/or laboratory values which would be considered medically notable (Section 8.1.1) and any events considered DLTs (Section 8.4.5). As such, the detailed summary tables for vital signs and in particular laboratory analytes will be part of the traditional safety assessment (Section 10.4.4) and referred to in the primary section. This would allow the CSR to present the integrated safety and efficacy information more succinctly.

Tumor response will be assessed and the changes between Baseline and pre-Prostatectomy evaluated and tabulated for the following:

- Gleason score of dominant lesion as determined from biopsy samples.
- Proportion of dominant lesion designated as adenocarcinoma as determined from biopsy samples.
- Proportion of subjects with local invasion as measured by mpMRI.

10.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Further evaluation of tumor response will be assessed and the changes between Baseline and pre-Prostatectomy evaluated and tabulated for the following:

- Change in volume of dominant lesion as measured by mpMRI.
- Change in PSA Density
- Change in PI-RADS Score

To address the additional secondary objective of the concentration of paclitaxel in the systemic circulation, concentration data collected as outlined below will, at a minimum be listed:

- PK samples taken at 1 and 2 hours post-injection at the first injection, and pre-injection, 1 and 2 hours post-injection at second and/or third injection visits, and at all other study visits
- Presence or absence of ejaculate paclitaxel concentration data for Days 15, 43, 57, and end of study Day 85.
- Presence/absence of paclitaxel in the tumor within the prostate, the ipsilateral lobe of the prostate, the contralateral lobe of the prostate, and pelvic lymph nodes.

10.4.4 SAFETY ANALYSES

Although the focus of the safety assessment is the interpretation of the AEs as these would contain any relevant changes throughout the study which could be seen as a safety signal, the laboratory data, vital signs will be presented as traditional safety displays.

Adverse events will be coded using the MedDRA dictionary. The events will be summarized by frequency and subset by grade/severity and relatedness. Serious AEs and DLTs will be highlighted with a focus on time to onset and duration. Events of special interest will be noted in the SAP and presented separately. It is anticipated that the AEs will also be presented by injection number and any AEs related to the injection process highlighted. These decisions will be made after the accumulating data is reviewed by the medical monitor. All decisions will be recorded in the SAP.

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 Screening to Day 85. 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).

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.

10.4.7 PLANNED INTERIM ANALYSES

An interim analysis is not planned as inferential analyses are not part of the analysis plan.

10.4.7.1 SAFETY REVIEW

The Medical Monitor will monitor safety throughout the study (Section 8.6). Therefore, no additional safety review is planned.

10.4.7.2 EFFICACY REVIEW

There are several assessments which will provide some preliminary efficacy information, and details are included in Sections 10.4.2, 10.4.3, and 10.4.11.

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 CSR as outlined in the ICH E3 guidelines.

10.4.11 EXPLORATORY ANALYSES

The changes in the PROs from Baseline to Day 85 will be summarized for the SHIM, the I-PSS and the NIH-CPSI and reviewed to provide insight on the possible changes in well-being.

IHC will be performed on slides obtained from the tumor excised at prostatectomy, and where available on slides from the ipsilateral and contralateral lobes of the prostate, and on a subset of lymph nodes excised during prostatectomy, and the data will be tabulated (as possible).

The FCM data will be reviewed on a quarterly basis. At a minimum all data collected will be listed in the final report.

10.4.12 CONCOMITANT MEDICATION

All medication taken during the trial will be, at a minimum, listed with the start and stop date, and will be coded using the World Health Organization (WHO) Drug Dictionary.

10.5 SAMPLE SIZE

The trial has been designed to assess the safety of the 15 mg/mL dose injected three times across an 85-day timeframe. The procedure nQuery Advisor version 8, POC 2-1 confidence interval for probability of observing a rare event, indicates that with 18 subjects the probability of detecting an event that occurs with a base rate of 0.1 is 85.0%, with a base rate of 0.05 is 60.3% and for a base rate of 0.01 is 16.5%.

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 subject are based. They will be separate and distinct from the eCRFs. These records should include detailed notes on:

- Medical history prior to the subject's involvement in the study;
- Date of informed consent;
- Basic identifying information that links the subect'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 subject;
- Medical condition during the subject's involvement in the study;
- All Adverse Events;
- Subject's exposure to the study agent;
- Subject's exposure to any concomitant therapy;
- All relevant observations and data on the condition of the subject throughout the trial;
- Justification for all entries in the subject'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 PI 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 5.0 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 subject confidentiality even with the data being stored in a US data center. The data management and statistical clinical research organization (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 ensure 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 subject 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 subject enrolled in the study, in accordance with the U.S. 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 the 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 subject 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 subject 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 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 data is ALCOA-C compliant.

For electronic source, the institution must provide a secure, validated electronic medical record (EMR) data management system that is 21 CFR Part 11 compliant and meets all regulatory requirements, regulations and quality standards.

For paper source, documentation is expected to be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies.

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 subject's official electronic study record.

The EDC application being used in this study is TrialMaster version 5.0 from OmniComm Systems (see Section 12 Quality Assurance and Quality Control).

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 (R2):

- 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.

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 subjects or participants, including PK measures and AEs. 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

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 PI, clinical trial site, and subjects.

18 LITERATURE REFERENCES

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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 penetration (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 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

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: NIH CHRONIC PROSTATITIS SYMPTOM INDEX (NIH-CPSI)

Pain or Discomfort

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

	Yes	No
a. Area between rectum and testicles (perineum)	☐ 1	☐ 0
b. Testicles	☐ 1	☐ 0
c. Tip of the penis (not related to urination)	☐ 1	☐ 0
d. Below your waist, in your pubic or bladder area	☐ 1	☐ 0

2. In the last week, have you experienced:

	Yes	No
a. Pain or burning during urination?	☐ 1	☐ 0
b. Pain or discomfort during or after sexual climax (ejaculation)?	☐ 1	☐ 0

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

☐ 0	Never
☐ 1	Rarely
☐ 2	Sometimes
☐ 3	Often
☐ 4	Usually
☐ 5	Always

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

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
0	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?

☐ 0	Not at all
☐ 1	Less than 1 time in 5
☐ 2	Less than half the time
☐ 3	About half the time
☐ 4	More than half the time
☐ 5	Almost always

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

☐ 0	Not at all
☐ 1	Less than 1 time in 5
☐ 2	Less than half the time
☐ 3	About half the time
☐ 4	More than half the time
☐ 5	Almost always

Impact of Symptoms

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

☐ 0	None
☐ 1	Only a little
☐ 2	Some
☐ 3	A lot

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

☐ 0	None
☐ 1	Only a little
☐ 2	Some
☐ 3	A lot

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?

☐ 0	Delighted
☐ 1	Pleased
☐ 2	Mostly satisfied
☐ 3	Mixed (about equally satisfied and dissatisfied)
☐ 4	Mostly dissatisfied
☐ 5	Unhappy
☐ 6	Terrible

Scoring the NIH-Chronic Prostatitis Symptom Index Domains

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 = _____

APPENDIX E: CYP3A4 SUBSTRATES, INDUCERS AND INHIBITORS

This is not an inclusive list. Because the lists of these agents are constantly changing, it is important to regularly consult frequently updated medical references.

CYP3A4 substrates	Strong Inhibitors[1]	Moderate Inhibitors	Weak Inhibitors	Inducers
alfentanil[4,5]	atazanavir	aprepitant	alprazolam	armodafinil
amiodarone[4]	boceprevir	conivaptan	amiodarone	barbiturates
aprepitant[5]	clarithromycin	crizotinib	amlodipine	bosentan
atorvastatin[5]	cobicistat	diltiazem	atorvastatin	carbamazepine
benzodiazepines	darunavir	dronedarone	bicalutamide	deferasirox
bortezomib	delavirdine	erythromycin	cilostazol	echinacea
brentuximab	grapefruit[3]	fluconazole	cimetidine	efavirenz
budesonide[5]	grapefruit juice[3]	fosamprenavir	ciprofloxacin	etravirine
buspirone[5]	indinavir	grapefruit[3]	cyclosporine	fosphenytoin
calcium channel blockers	itraconazole	grapefruit juice[3]	fluvoxamine	glucocorticoids[2]
cisapride	ketoconazole	imatinib	fosaprepitant	modafinil
citalopram/escitalopram	lopinavir/ritonavir	mifepristone	isoniazid	nafcillin
conivaptan[5]	nefazodone	nilotinib	nicardipine	nevirapine
glucocorticoids[2]	nelfinavir	verapamil	propofol	oxcarbazepine
crizotinib	posaconazole		quinidine	phenobarbital
cyclosporine[4]	ritonavir		ranolazine	phenytoin
cyclophosphamide	saquinavir			pioglitazone
dapsone	telaprevir			primidone
darifenacin[5]	telithromycin			rifabutin
darunavir[5]	voriconazole			rifampin
dasatinib[5]				rifapentin
dihydroergotamine[4]				ritonavir
docetaxel				St. John's wort
doxorubicin				topiramate
dronedarone[5]				
eletriptan[5]				
ergotamine[4]				
eplerenone[5]				
erlotinib				
esomeprazole				
estrogens				
etoposide				
everolimus[5]				
felodipine[5]				
fentanyl[4]				
fosaprepitant[5]				
gefitinib				
haloperidol				
HIV antiretrovirals				
HMG Co-A inhibitors[5]				
ifosfamide				
imatinib				
indinavir[5]				
irinotecan				

CYP3A4 substrates	Strong Inhibitors[1]	Moderate Inhibitors	Weak Inhibitors	Inducers
itraconazole ketoconazole lansoprazole lapatinib losartan lovastatin[5] lurasidone[5] macrolide antibiotics maraviroc[5] medroxyprogesterone methadone midazolam[5] modafinil montelukast nefazodone nilotinib nisoldipine[5] omeprazole ondansetron paclitaxel pazopanib quetiapine[5] quinidine[4] saquinavir[5] sildenafil[5] simvastatin[5] sirolimus[4,5] sunitinib tacrolimus[4,5] telaprevir tamoxifen temsirolimus teniposide tetracycline tipranavir[5] tolvaptan[5] triazolam[5] trimethoprim vardenafil[5] vinca alkaloids zolpidem				

[1] Certain fruits, fruit juices and herbal supplements (star fruit, Seville oranges, pomegranate, gingko, goldenseal) may inhibit CYP 3A4 isozyme, however, the degree of that inhibition is unknown.
[2] Dexamethasone is considered a weak CYP3A4 inducer.
[3] The effect of grapefruit juice (strong vs moderate CYP3A4 inhibition) varies widely among brands and is concentration-, dose-, and preparation-dependent.
[4] Narrow therapeutic range substrates
[5] Sensitive substrates
Websites: *https://www.pharmacytimes.com/publications/issue/2008/2008-09/2008-09-8687;*
https://www.ebmconsult.com/articles/medications-inhibitors-cyp3a4-enzyme