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TITLE: Randomized trial of image -guided stereotactic radiation therapy (IG-SRT) in prostate cancer

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Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCMC.

List of Abbreviations

All abbreviations used throughout the protocol must be defined.

AE	Adverse Event
CBC	Complete Blood Count
CBCT	Cone Beam Computed Tomography
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTV	Clinical Target Volume
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
DVH	Dose Volume Histogram
EPIC	Expanded Prostate Cancer Index Composite Instrument
ERB	Endorectal Balloon
FDA	Food and Drug Administration
Fx	Fraction
GCP	Good Clinical Practice
GI	Gastrointestinal
GTV	Gross Target Volume
Gy	Gray
GU	Genitourinary
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRBFA	Human Research Billing Analysis Form
HRQOL	Health-related Quality of Life
ICF	Informed Consent Form
IG	Image Guided
IMRT	Intensity Modulated Radiation Therapy
IND	Investigational New Drug
IRB	Institutional Review Board
NCCN	National Comprehensive Cancer Network
PEG	Polyethylene Glycol
PHI	Protected Health Information
PI	Principal Investigator
PSA	Prostate Specific Antigen

PTV	Planning Target Volume
REDCap	Research Electronic Data Capture
RS	Rectal Spacer
RT	Radiation Treatment
SAE	Serious Adverse Event
SBRT	Stereotactic Body Radiation Therapy
SUSAR	Suspected Unexpected Serious Adverse Reaction
TRUS	Transrectal Ultrasound
UAP	Unanticipated Problem
WCMC	Weill Cornell Medical College

Protocol Summary

Randomized trial of image -guided stereotactic radiation therapy (IG-SRT) in prostate cancer

IRB Protocol #:

Short Title:	Randomized trial of image-guided prostate SRT
Principal Investigator:	Dr. Josephine Kang
Sample Size:	N = 40
Accrual Ceiling:	This study plans to enroll a total of 40 patients
Study Population:	Patients with low-risk or favorable intermediate-risk prostate cancer as defined by 1.2016 NCCN criteria
Accrual Period:	4 years
Study Design:	Randomized, two-arm study. Patients will be randomized to either rectal spacer placement or endorectal balloon placement, daily prior to each radiation treatment.
Study Duration:	5 years
Study Agent/ Intervention Description:	

1. Endorectal balloon (ERB): Immobilization device manually placed into the rectum prior to radiation treatment planning CT and daily treatment delivery, to immobilize the prostate and reduce prostate motion.

2. Rectal spacer (RS): Biodegradable gel that is transperineally injected between the rectum and prostate under transrectal ultrasound guidance, to increase physical distance and thereby reduce radiation dose to the anterior rectal wall. The spacer begins to biodegrade in 2-3 months, and is fully absorbed within 6 months.

Primary Objective:

1. To compare acute GI/GU toxicity (as defined by CTCAE v4.0)
2. To compare rectal dose (V35, max rectal dose)

Secondary objective:

1. To compare health-related quality of life (HRQOL) measured using the Expanded Prostate Cancer Index Composite (EPIC) instrument for bowel, urinary and sexual domains
2. To compare biochemical recurrence-free survival at 1, 2 and 5 years
3. To compare the dose distribution of the 2 techniques, specifically:
   - **a)** coverage of the PTV
   - ***b)*** DVH of organs at risk (OAR)

c) prostate motion and shifts required during treatment

4. To compare late GI/GU toxicity (as defined by CTCAE v4.0)

Exploratory Objectives:
1. To explore microbiome changes associated with normal tissue toxicities resulting from radiation
2. To collect complete blood count (CBC) measurements at pre-treatment, 1, 3 , 6 and 12 months post-treatment to assess impact of SBRT, if any, on baseline values

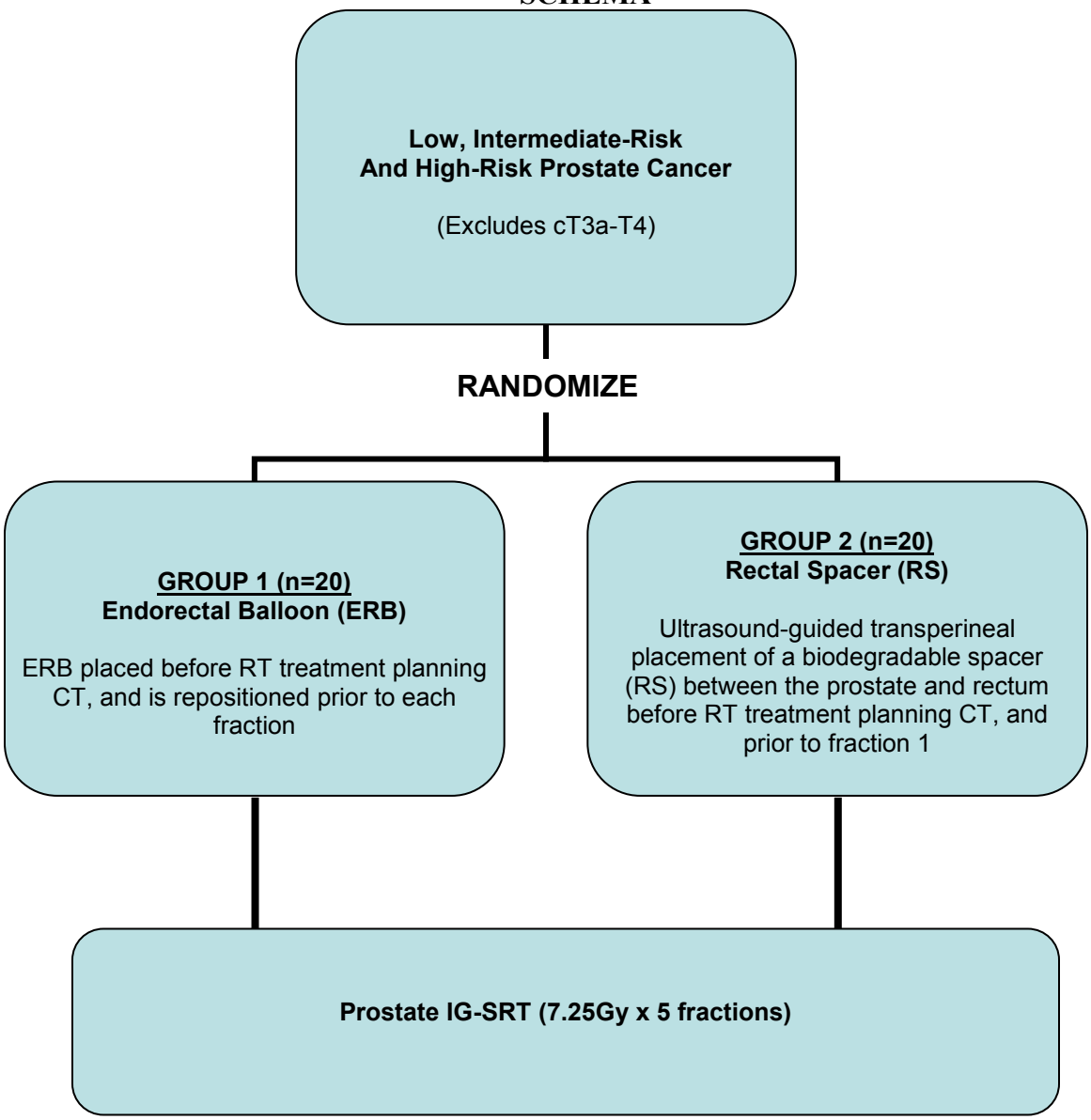
Endpoints:
The primary endpoints of this study are

a) Acute GI/GU toxicity
b) Rectal dose (Dmax, V35)

The secondary endpoints of this study are:

a. EPIC bowel, urinary and sexual domain outcomes
b. Biochemical recurrence-free survival
c. Late GI/GU toxicity
d. Dose distribution
e. Microbiome alterations
f. CBC measurements

SCHEMA



Follow-up visits at 3-4, 12 and 24 weeks, then q6 months
At each follow-up: CTCAE toxicity assessment, EPIC evaluation, PSA measurement
Microbiome sample collection: during CT simulation, during first RT treatment and last RT treatment, and at first post-RT follow up.

14 CBC collection: pre-RT, and at each post-RT follow-up for up to 1 year.

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1.0 STUDY OBJECTIVES

HYPOTHESIS: A rectal spacer will reduce rectal radiation, compared to endorectal balloon, and reduce the incidence of acute and late toxicities of IG-SRT to the prostate, with comparable QOL and tumor control results

1.1. Primary Objectives

1. To compare acute GI/GU toxicity (as defined by CTCAE v4.0)
2. To compare rectal dose (V35, max rectal dose)

1.2 Secondary Objectives

1. To compare health-related quality of life (HRQOL) measured using the Expanded Prostate Cancer Index Composite (EPIC) instrument for bowel, urinary and sexual domains
2. To compare biochemical recurrence-free survival at 1, 2 and 5 years
3. To compare the dose distribution of the 2 techniques, specifically:
    - **a)** coverage of the PTV
    - ***b)*** DVH of organs at risk (OAR)
    - ***c)*** prostate motion and shifts required during treatment
4. To compare late GI/GU toxicity (as defined by CTCAE v4.0)

1.3 Exploratory Objectives

1. To explore microbiome changes associated with normal tissue toxicities resulting from radiation
2. To collect complete blood count (CBC) measurements at pre-treatment, 1, 3 , 6 and 12 months post-treatment to assess impact of SBRT, if any, on baseline values

2 BACKGROUND

2.1 Prostate cancer radiotherapy

Other than skin cancer, prostate cancer is the most commonly diagnosed cancer in men, and second leading cause of cancer death. [1] It is estimated that one out of seven men will be diagnosed with prostate cancer during their lifetime. The average age at diagnosis is 66. Due to the availability of prostate specific antigen (PSA) testing, the majority of men are diagnosed with organ-confined prostate cancer, which is further stratified by NCCN into low-, intermediate- and high-risk disease based on T-stage, initial PSA, and Gleason score. Intermediate-risk prostate cancer is classified as favorable versus unfavorable, depending on the number of intermediate-risk group features.[2]

Patients with low- and favorable intermediate-risk prostate cancer have multiple treatment options available, including surgery (e.g., radical prostatectomy ± pelvic lymph node dissection), brachytherapy or external beam radiation (EBRT). EBRT is the least invasive of the

aforementioned options. Standard EBRT is delivered over a time course of 9-10 weeks, and entails daily treatment of up to 50 treatment fractions. Due to the protracted course of standard EBRT, hypofractionated approaches have been investigated, with promising results thus far. Hypofractionated EBRT delivers higher dose per each fraction, thereby allowing a decrease in the total number of fractions required. There is now data supporting use of extremely hypofractionated EBRT for high risk prostate cancer, showing comparable results to standard EBRT; [3-6]

Extremely hypofractionated regimens are delivered most commonly within 5 fractions, allowing completion of treatment within 1-2 weeks. Such treatments are delivered using stereotactic or image-guided IMRT approaches, and most commonly referred to as prostate SRT. Single institutional and pooled reports have demonstrated similar efficacy and toxicity to conventionally fractionated regimens. [3,6,7] The NCCN treatment paradigm currently includes SRT as an alternative to conventionally fractionated regimens at clinics with appropriate technology, physics and clinical expertise.[2]

2.2 Investigational Agent or Device

2.2.1 Endorectal balloon

The RectalPro endorectal balloon (ERB) by QLRAD, Inc. will be used for patients on the ERB arm. This ERB which has been utilized successfully in numerous studies without adverse outcomes.[8-10] The ERB is an inflatable device, typically filled with approximately 60 ml of air or sterile water and introduced into the rectum to a set depth (most common: 12 cm) with locking stopper to fix positioning. [11] Endorectal balloons have been utilized during prostate radiation to reduce intrafraction prostatic motion, and improve reproducibility. Multiple published studies have reported significant decrease in prostate motion with use of a rectal balloon during 3-D conformal radiation as well as intensity-modulated radiation therapy (IMRT). [8,12-14] Studies have shown that ERB can be successfully used during prostate SRT to both reduce prostatic motion and improve set up reproducibility.[11,15]
The ERB will be placed into the rectum prior to the radiation treatment planning CT, and prior to each radiation treatment. The balloon is promptly removed immediately upon completion of each radiation treatment. No changes to the device will occur.

2.2.2 Rectal spacer

The SpaceOAR gel system will be utilized for all patients on the rectal spacer arm. This is an FDA-approved, biocompatible, absorbable hydrogel that will be injected transperineally with transrectal ultrasound (TRUS) guidance and local anesthesia to create a distance of 1.0-1.5 cm between the rectum and prostate. According to a multicenter, randomized trial, use of the SpaceOAR rectal spacer increased the mean perirectal space to 12.6 + 3.9 mm, compared to 1.6 + 2.0 mm in the control arm.[16] Numerous studies have shown this procedure to be safe and well-tolerated, with minimal risk of side effects.[17-20] Approximately 3 months after injection, the hydrogel begins to liquefy and is slowly absorbed into the system and cleared via renal filtration.

The majority of published studies have looked at use of rectal spacers prior to standard fractionation external beam radiation and brachytherapy. [17-20] A prospective randomized study of 222 patients has demonstrated significant reduction in late bowel toxicity with use of rectal spacers. In this study, patients were randomized to hydrogel rectal spacer versus no spacer prior to undergoing fiducial-guided IGRT to dose of 79.2 Gy No device-related adverse events, infection or serious bleeding were reported.[16] There was a significant decrease in late rectal toxicity (7% vs 2%), and decreased likelihood of experiencing a 10-point decline in bowel quality of life EPIC score (12% vs 21%). By using a spacer, the volume of rectum receiving 70 Gy or higher decreased from 12% to 3%, allowing the rectal wall to be excluded from exposure to high radiation doses in majority of patients. A comparable endpoint for 5-fraction SBRT, is the volume of rectum receiving >35 Gy , a parameter we selected as a primary study endpoint.

2.3 Rationale

Stereotactic radiation therapy (SRT) delivers high doses of radiation to the prostate, most commonly 36.25 Gy, in 5 fractions over 1-2 weeks. Single-institution studies and pooled analyses demonstrate excellent outcomes, comparable to standard EBRT which requires up to 10 weeks of daily treatment.[3,6] Late grade 3 genitourinary and gastrointestinal toxicity also appears to be comparable to standard EBRT, ranging from 0.6 – 3% [3,6] with longest median follow-up of 7 years.

During radiation treatment planning, the prostate volume is expanded several millimeters to create the planning target volume (PTV). The prostate is situated immediately anterior to the rectum. As a result, the rectal wall is almost always included in the target volume, and becomes the major dose-limiting organ. Studies on conventionally fractionated radiation and brachytherapy have demonstrated that increased dose to the rectum is directly correlated with increased risk of rectal toxicity.[21-23] It is equally important to avoid high dose to the urethra, which runs centrally through the prostate, and be cognizant of dose to the bladder neck, which overlies the superior/anterior aspect of the prostate. Meticulous treatment planning techniques are required to accurately identify the prostate, and avoid depositing high dose to normal structures, particularly during SRT since the dose per fraction is much higher than EBRT.

SRT delivers high dose to the target with a rapid dose fall-off. Thus, it is important to limit prostate motion as much as possible during treatment delivery. A small shift in the prostate can result in decreased dose to the target. There are multiple techniques to account for prostate motion during delivery of SRT. Such techniques include tracking via implanted radiofrequency transponders (e.g., Calypso), on-board kV imaging of prostate fiducials at 30-60 second intervals (e.g., Cyberknife), and use of multiple cone-beam CTs during treatment.

Physical devices such as the ERB have shown promise in reducing prostate motion during treatment.[13,14] By placing the ERB directly into the rectum and securing it in place, the prostate is displaced anteriorly, limiting posterior shifts. Studies have demonstrated that ERB improves rectal dosimetry, by distancing the posterior and lateral rectal wall from the radiation target and thereby sparing these regions of the rectum from excessive exposure to radiation.[12,13] However, at least one study examining use of ERB during SRT suggests the reduction in radiation dose to

the posterior and lateral rectal wall results in higher dose to the anterior rectal wall.[11] Increase in rectal wall dose has also been demonstrated for EBRT.[14] Whether increased anterior rectal wall dose translates to a clinically meaningful detriment to acute and late GI toxicity remains unknown at this point in time, and further studies are needed.

Rectal spacers are being utilized with increasing frequency to reduce dose to the rectum, but studies examining use of rectal spacers in SRT are limited. By inserting a rectal spacer between the prostate and rectum, a physical distance is constructed between the posterior aspect of the prostate and anterior rectal wall. As a result, a high dose can be delivered safely with standard margins, while sparing normal tissue. According to one study, after rectal spacer placement, a safety margin of 4 mm from CTV to PTV appears to be sufficient to cover all prostate movements, **even without any correction**. This is in contrast to motion studies on patients undergoing radiation without spacer placement, who have demonstrated intrafraction motion of up to 9 mm over the course of 8-16 minutes.[24] Thus, there is suggestion that a spacer may act as a stabilizer.[25] As a result, it is possible that use of a rectal spacer can limit prostate motion, while protecting the rectum. By reducing rectal dose, use of a rectal spacer may result in decreased acute and late toxicity.

Our study seeks to help address this hypothesis. Despite the variety of accepted techniques for prostate SRT delivery, to our knowledge there are no studies comparing dosimetric endpoints, toxicity and HRQOL endpoints after randomization to two different methods. Additional studies are needed to determine whether rectal spacers are superior to endorectal balloons.

During SRT planning, increased dose heterogeneity allows improved dose fall-off at target boundaries, whereas improved dose homogeneity results in a slower dose fall-off gradient. For situations where the target is in close proximity to a normal organ, greater dose heterogeneity can be accepted in order to protect the normal organ. For prostate SRT with ERB, maximizing dose fall-off at the prostate-rectal wall interface may result in regions of high-dose deposition within the target volume, and care must be taken to avoid high-dose to the urethra, which lies within the target. On the other hand, delivery of prostate SRT with a rectal spacer allows dose homogeneity to be prioritized, [26] since dose fall-off at the target boundary is less of a concern due to the distance between the prostate and rectal wall. As a result, we hypothesize that a more homogeneous dose distribution to the prostate, with improved target coverage, can be achieved by using a spacer versus rectal balloon, while at the same time reducing dose to the rectal wall and avoiding urethral hotspots. Our primary endpoint will be dose to the rectum, measured as rectal Dmax (maximum dose received to the rectum) and rectal V35 (volume of rectum receiving 35 Gy or more), and associated acute GI/GU toxicity. As secondary endpoints, we will assess late toxicity, EPIC HRQOL,biochemical recurrence free survival and compare dose-volume histograms of organs at risk (rectum, bladder), intra-fraction prostate motion.

2.4 Risk/Benefit Assessment

2.4.1 Endorectal Balloon:

There are no documented reports of any significant complications resulting from ERB placement. We anticipate that the ERB will be in place for approximately 30-45 minutes during treatment planning CT, and approximately 10-15 minutes during each of the 5 radiation treatments. It is expected that ERB placement in the rectum may result in a sensation of pressure or discomfort, but there should not be any pain during or after the procedure. Any discomfort/pressure from the rectal balloon should resolve once the balloon is removed.

2.4.2 Rectal Spacer:

There are numerous reports on use of hydrogel rectal spacers during prostate RT, with no documented reports of any significant toxicity or complications. Spacers are FDA-approved, biocompatible, non-toxic and non-allergenic, and insertion is well-tolerated in published reports.[27] Prior to spacer placement, lidocaine is injected into the perineal area to provide local anesthesia. There may be slight discomfort associated with the local anesthesia, and sensation of pressure during the rectal spacer placement. At time of spacer injection, 4 gold fiducial markers are introduced into the prostate for localization, a standard procedure prior to image-guided RT for prostate cancer. The risks of spacer placement are minimal, and include possible bleeding/hematoma formation, infection, and skin irritation.

A potential benefit of using a rectal spacer includes improved dosimetry and decreased radiation dose to surrounding rectum, bladder neck and urethra. This may potentially translate into late acute- and long-term toxicity. Also, if our hypothesis is correct, that use of a spacer allows homogeneous dose distribution with better PTV coverage, it is possible that biochemical recurrence-free survival also improves as a result.

2.5 Correlative Studies Background

2.5.1 Microbiome sampling.

During radiation treatment for prostate cancer, the anterior rectal wall will receive a high dose of radiation. As a result, the rectal mucosa will undergo signs of radiation injury, characterized by inflammation as well as loss of the epithelial barrier.[28,29] Clinically, mucositis manifests as bloating, rectal discomfort and loose stools.[30,31] Recent studies have demonstrated a role for commensal intestinal microbiota on development and severity of mucositis. It has been suggested that certain microbiota predispose for radiation-induced diarrhea, but there are very few studies on this.[32] To our knowledge, there are no studies on microbiome changes after exposure to ablative doses of radiation, and the correlation with rectal mucosa toxicity.

Patients undergoing radiation for prostate cancer require digital rectal examination as part of routine physical and follow up visits. During this process, it is simple to acquire a small fecal sample to process. We plan to obtain samples at the time of the radiation planning CT, after the

first and last radiation treatment, and at the first follow up visit. If the patient prefers, he will have the option of providing us with a stool sample.

2.5.2 CBC assessment

Radiation beams pass through the pelvic bone marrow. The acute and long-term impact on the white blood cells has not been well-studied. Since patients undergoing prostate radiation undergo routine blood tests, including CBC, we plan to record this data up to 12 months post-RT for our analyses. We hypothesize that there will be minimal impact on blood counts after prostate SBRT.

3. SUBJECT SELECTION

3.1 Study Population

Men with a histologically confirmed diagnosis of low-, intermediate- or high-risk prostate adenocarcinoma, meeting the inclusion and exclusion criteria below, and electing to undergo definitive radiation treatment, will be eligible for participation in this study. Use of androgen deprivation therapy (ADT) will be left to the discretion of the treating physician(s).

3.2 Inclusion Criteria

1. Biopsy-proven diagnosis of prostate adenocarcinoma, diagnosed within 1 year of randomization
2. Either NCCN-defined low-risk disease (T1c-T2a, Gleason score 3+3=6, PSA <10), intermediate-risk disease (Gleason score 3+4=7, 4+3=7, T2b-c and/or PSA 10-20;) or high-risk disease due to Gleason score 8-10 and/or PSA >20 ng/ml, but not due to T3-T4 disease on physical exam.
3. Age ≥ 18

3.3 Exclusion Criteria

1. History of prior pelvic radiation (external beam or brachytherapy)
2. Prior or concurrent lymphomatous/hematogenous malignancy, or history of prior/concurrent invasive malignancy during the past 5 years
3. Very high risk prostate cancer (T3b-T4 on clinical exam, Primary Gleason pattern 5, or >4 cores with Gleason score 8-10)
4. History of prior chemotherapy for prostate cancer
5. History of irritable bowel disease
6. Evidence of lymph node involvement
7. AUA score >15
8. Prostate size > 90 cc

4 REGISTRATION PROCEDURES

4.1 Patient Registration

Before any protocol specific procedures can be carried out, investigators/staff will fully explain the details of the protocol, the study procedures and the aspects of patient privacy regarding research information. Patients will be provided a comprehensive explanation of the proposed treatment including the type of therapy, the rationale for treatment on the protocol, alternative treatments that are available, any known adverse events, the investigational nature of the study and the potential risks and benefits of the treatment. The informed consent document will meet all requirements of the Institutional Review Board (IRB). All subjects/patients are informed in the consent that participation or refusal to participate in the research study will not affect any of the clinical treatment or services to which they would otherwise be entitled.

The physicians who may obtain informed consent are listed on the title page of this protocol. The informed consent form will be signed by the participant and the registering physician. Once signed, a copy will be given to the patient and one will be maintained with the patient's medical record. Once eligibility is confirmed and informed consent is documented, the patient will be registered by the study coordinator/data manager.

Patients will be centrally registered with the Office of Billing Compliance. To register a patient, submit the following documents via the JIRA Registration Process:

- Legible copy of the HRBAF
- Signed informed consent

Registration must be completed within 24 hours of the signing of informed consent.

14 Study Procedures

Schedule of Evaluations

	Pre-Study	Planning CT	Fx 1	Fx 2	Fx 3	Fx 4	Fx 5	Post-RT Wk 3-4	3 mos	6 mos	12 mos	18 mos – 60 mos (assessment every 6 mos)
Rectal Spacer Placement	X											
Endorectal Balloon Placement		X	X	X	X	X	X					
Informed consent	X											
Demographics	X											
Medical history	X							X	X	X	X	X
Physical exam	X							X	X	X	X	X

CBC w/diff, plts	X							X	X	X	X	X
PSA	X									X	X	X
Acute/Late Toxicity Assessment (CTCAE)								X	X	X	X	X
EPIC Questionnaire	X							X	X	X	X	X
Microbiome sample collection		X	X				X	X				

5.1 Pre-Study Visit

At the initial screening visit, patient will undergo:

- Informed consent
- Medical history
- Medication History
- Physical Exam
- Baseline EPIC Questionnaire
- Routine labs (CBC), if not done within the past 4 weeks

Eligible subjects will be randomly assigned to endorectal balloon versus rectal spacer placement in a 1:1 ratio, using a computer-generated randomization scheme developed by the data manager.

Patients randomized to the rectal spacer arm will be sent to a urologist for rectal spacer placement and also, optionally, placement of 3-4 gold fiducial markers into the prostate, which is a standard procedure for many patients prior to prostate RT. This must occur at least 5 days prior to the planning CT and pelvic MRI.

Patients randomized to the ERB arm do can be scheduled for CT sim and MRI directly without any intervening procedures.

5.2 Planning CT/MRI Patients will be instructed to undergo a Fleet enema prior to planning CT. All patients will be sent for a pelvic MRI. The ideal MRI will be 3Tesla and in a position similar to treatment position, without use of an endorectal coil which can distort the prostate shape.

Patients who are on the rectal spacer arm should wait at least 5 days prior to MRI to allow for edema to resolve.

Treatment planning CT will be performed with vac loc immobilization. Patients will be advised to drink 1 cup of water 1 hour prior to the CT simulation to allow for a comfortably full bladder, if possible. A rectal catheter will be utilized to dispel any excess bowel gas. Patients randomized to the ERB arm will undergo CT sim with the ERB in place. Microbiome sample collection (stool from digital rectal exam will be collected)

5.3 Radiation Treatment Planning

5.3.1 Total Prescribed Dose

Patients on each arm will receive 5 fractions of radiation, 7.25Gy per fraction, delivered every day or every other day excluding weekends), to total dose of 36.25 Gy. .

5.3.2 Dose Coverage

For both arms, V36.25 Gy (volume of the PTV receiving 36.25 Gy) must be ≥ 95%

5.3.3 Radiotherapy Delivery

Radiation can be delivered using IMRT or VMAT technique. If using fixed gantry angle IMRT, at least 5 gantry positions must be used.

5.3.4 Image Guidance

a. Arm 1: Endorectal Balloon: Patients randomized to this arm will receive image guidance with cone beam CT (CBCT) prior to, and during, each radiation treatment. The CBCT image will be examined by the radiation oncologist to ensure that the prostate is in appropriate position, and necessary shifts, if required, will be made prior to and during treatment to account for both inter- and intrafraction motion.

b. Arm 2: Rectal Spacer: Patients randomized to this arm can be tracked either using 3D systems (CBCT), or 2D systems (ExacTrac, on board imaging, electronic portal imaging device, etc). Prostate position will be verified using the fiducials with 2 mm allowance around fiducial location at planning CT to fiducial location at time of actual treatment.

5.3.5 Treatment planning parameters

a. The clinical target volume (CTV) will consist of the prostate +/- proximal 1-2 cm of the seminal vesicles. This will be defined on the axial CT scan, with correlation to the pelvic MRI (fused, when possible, using the gold fiducials for patients on the rectal spacer arm).
b. The PTV will be defined as 5 mm isotropic margin with 3 mm posterior margin. If necessary to meet dose constraints, the superior and anterior margin can be reduced to 3 mm.
c. Normal tissues to contour: bladder, rectum, bilateral femoral heads, penile bulb, small bowel that is close to target volume. Rectum should be contoured from anus to the rectosigmoid flexure.
d. Patients on the rectal spacer arm will have the spacer delineated, based on MRI.

5.3.6 Dosimetry

a. Maximum dose allowed to PTV is 38.78 Gy (107% of prescription dose). At least 95% of the PTV must receive 36.25 Gy. No aspect of the PTV should fall below 34.4 Gy.
b. Critical organ limits:

1. Rectum: Maximum point dose 38.06 Gy (105% prescription dose), D1cc 38.5 Gy
2. Bladder: Maximum point dose 38.06 Gy (105% prescription dose)
3. Penile Bulb: No more than 100% of prescription dose; D3cc 20 Gy
4. Femoral heads: Maximum point dose 30 Gy
5. Small bowel: Maximum point dose 25 Gy

5.47 Radiation Treatment

Radiation treatments will be delivered on an outpatient basis. Approximately 2-3 hours before each radiation treatment session, the patient will undergo a fleet enema. On each treatment day, he will drink 1 cup of water approximately 1 hour before treatment time to allow bladder to be comfortably full, if patient can tolerate this.

a. ERB arm: Before treatment, the endorectal balloon (RectalPro by QLRAD, Inc) will be positioned and locked into place. CBCT will be done to verify position and reproducibility. A second CBCT will be performed in between the treatment to account for any intrafraction motion.

b. RS arm: Before treatment, CBCT will be performed to ensure the bladder is full to similar extent as time of simulation (this can impact seminal vesicle position), and also to look for excess gas in the rectum. If the rectum has excess gas, patient will be given the opportunity to dispel the gas or a rectal catheter can be temporarily placed to release the gas, and the catheter will be removed prior to start of treatment. Appropriate image guidance techniques will be utilized (please see 5.3.4 Image guidance).

5.5 Supportive Care Guidelines

a. Urinary: A proportion of patients undergoing prostate SRT can expect increase in urinary frequency or urgency. If this becomes bothersome to the patient, medication to alleviate symptoms can be prescribed at the discretion of the treating radiation oncologist and documented in patient chart.
b. Bowel: Bowel symptoms during time of prostate SBRT are rare. If patients develop rectal urgency, tenesmus or diarrhea, medication to alleviate symptoms can be prescribed at the discretion of the treating radiation oncologist, and documented in patient chart.

5.6 Duration of Therapy and Criteria for Removal from Study

In the absence of treatment delays due to adverse event(s), radiation treatment is anticipated to complete within 2 weeks time. Patients can be removed from the study at any point should they decide they no longer wish to participate. They will continue to receive routine medical care as necessary outside the confines of this study.

5.7 Duration of Follow Up

Patients will be followed for after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6 DOSE MODIFICATIONS

None.

7. ADVERSE EVENT REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug or device under investigation. Safety will be monitored by evaluation of adverse events reported by patients or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x-rays, electrocardiographs, etc.

7.1 Adverse Event Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

Adverse Event Characteristics and Related Attributions

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.2 Recording of Adverse Events

All adverse events will be recorded on a patient specific AE log. The AE log will be maintained by the research staff and kept in the patient's research chart.

7.2.1 Reporting of AE to WCMC IRB

All AEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

7.3 Definition of SAE

SAE's include death, life threatening adverse experiences, hospitalization or prolongation of hospitalization, disability or incapacitation, overdose, congenital anomalies and any other serious events that may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.3.1 Reporting of SAE to IRB

All SAEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

7.3.2 Expedited Adverse Event Reporting

The principal investigator is responsible for monitoring the safety of patients who enroll in the study. All AEs occurring after any administration of the study drug will be followed until resolution. The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be used for adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

A serious adverse event (SAE) is any adverse drug experience that occurs at any dose that results in any of the following outcomes:

- Death.
- Life-threatening adverse drug experience.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- For the purpose of this study, hospitalizations for protocol-scheduled procedures, blood product transfusions, or for social reasons (i.e., awaiting transport home) will not be considered SAEs.
- Persistent or significant disability/incapacity.
- A congenital anomaly/birth defect.

- Important medical events: Defined as AEs that, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent 1 of the outcomes listed above, even though these events may not be immediately life-threatening or result in death or hospitalization.

All SAEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

7.3.3 AE/SAE Follow Up

All SAEs and AEs reported during this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized and no more follow-up is required. This requirement indicates that follow-up may be required for some events after the patient discontinues participation from the study.

8 PHARMACEUTICAL INFORMATION

There is no investigative agent used on this protocol.

9 CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Microbiome collection

9.1.1.1 Collection of Specimen(s): Fecal samples will be obtained via digital rectal exam and placed into an eppendorf tube. Alternatively, patients will have the option of providing stool samples from home into a plastic container.

9.1.1.2 Handling of Specimens(s): Samples will be promptly frozen at 20 degrees C, and transferred within the next week to a -80 degree freezer or liquid nitrogen for long-term storage. DNA extractions and 16sRNA analysis will be performed once all samples are collected.

The microbial DNA will be isolated and used to provide DNA sequence information. We will perform taxonomic characterization of bacteria (using 16S rRNA) and fungi (using ITS sequences). Prokaryotic diversity will be screened using massively parallel DNA sequencing, exploiting a multiplexing technique to generate 16S rRNA sequence tags, followed by analyses (statistical, clustering, and phylogenetic) to estimate the distribution of phylotypes, differential abundance, and the relative contributions between phylotypes and community dissimilarities to the overall diversity in individuals.

In parallel studies, potential relationships with the fungal community will be characterized. An important consideration in microbiome research is the optimal level (e.g. phylum, genus, species, strain, gene) at which to examine a scientific question [33-36]. We will therefore also examine whether there are metagenome sequence content differences in patients displaying acute/late rectal mucosal toxicity versus those who remain unaffected. High-throughput sequencing will be performed for transcriptome analysis.

We will examine whether there are metagenome sequence content changes during radiation treatment, and study whether differences in gene content indicate differences in functional pathways between the normal and irradiated microbiome using pathway analysis.

Samples will be collected by the research team and the samples will be stored in -80C freezer in Dr. Silvia Formenti's lab until we establish a lab for sample analysis.

9.1.2 Coding of Specimens

All patients enrolled will be given a unique identifier (study ID number). Only the data manager will know the code linking patient and study ID number. Patients will be assigned a unique code number. All specimens collected will be de-identified and assigned the same unique study number of the corresponding patient and will also be marked with the collection time point. Clinical information regarding toxicities and response will likewise be stored in a de-identified database using only the unique identifier (study ID number).

10 MEASUREMENT OF EFFECT

10.1 Biochemical Recurrence-Free Survival

Biochemical recurrence-free survival will be measured using the Phoenix criterion of PSA nadir + 2 ng/mL as evidence of biochemical failure.

11 DATA REPORTING / REGULATORY CONSIDERATIONS

11.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled patients.

11.1.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

11.1.2 Regulatory Considerations

All protocol amendments and consent form modifications will be made by the Principal Investigator.

11.1.3 Data Management

All patient data will be entered and maintained in REDCap. These data include clinical data and all patient safety data. The REDCap provides audit trails that track creation and modification of records that include user id and timestamp. Once entered, the data is subjected to validation procedures that are executed either immediately or upon saving the eCRF page or during the batch validation process. Validation failures that are identified before the page is saved can be corrected immediately. Validation failures during saving of the eCRF page and during batch validation processes will generate a discrepancy. Depending on the database account privileges, the data managers may be able to correct a discrepancy or if not, route it to the project data manager at WCMC who can take appropriate action to correct the problem. Data clarification forms can also be printed out when necessary to be sent to the project data manager at JCTO. Once the discrepancy is closed, by marking "resolved" or "irresolvable", the data is marked clean and an audit trail is generated by the system.

All key end points will be source verified by a second person at each site and errors will be corrected. Once the data is verified and all discrepancies are closed, the data can be locked/frozen. Locking and freezing can be done at different granular levels and will follow institutional SOPs and any specific requirements for the project.

Security measures that will be taken in order to protect patient data will include firewall technology and database level security which will be achieved by assigning roles and privileges to different levels of users and by requiring that the users authenticate themselves using user id and password. Additional security for data transfer between remote clients and servers will be achieved by using digital certificates/SSL. All data will be backed-up to tape periodically according to the Institutional SOPs. All data will be stored for at least 5 years following the termination of this study.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

The primary endpoints of this study are

a) Acute GI/GU toxicity
b) Rectal dose (Dmax, V35)

The secondary endpoints of this study are:

a. EPIC bowel, urinary and sexual domain outcomes

b. Biochemical recurrence-free survival
c. Late GI/GU toxicity
d. Dose distribution
e. Microbiome alterations
f. CBC measurements

12.2 Sample Size/Accrual Rate

The primary aim of this study is to compare the acute GI/GU toxicity proportion between patients receiving the endorectal balloon (Group 1) and patients receiving the rectal spacer (Group 2). With 20 patients in Group 1 and 20 patients in Group 2, a two group chi-square test with a 0.05 two-sided significance level will have 80% power to detect the difference between a endorectal balloon (Group1) acute grade I/II GU toxicity proportion of 72% and a rectal spacer (Group 2) acute grade I/II GU toxicity proportion of 29% (i.e., 60% reduction). Assuming acute grade I/II GU toxicity proportions of 72% and 29% in Groups 1 and 2, respectively, a 95% confidence interval for the difference in the toxicity proportion (43%) can be constructed to be within ± 27.9% of the observed difference in the toxicity proportion between Groups 1 and 2. This same calculation would also apply to acute grade I/II GI toxicity, which is hypothesized to be 77% in Group 1 and 31% in Group 2 (i.e., 20 patients per group would provide 86% power for this GI toxicity comparison with a two-sided alpha level of 5%).

Randomization:

A series of randomized blocks of 4 will be generated with a 1:1 allocation ratio. This will provide assurance that after four patients are enrolled in the study, there will be two patients assigned to Group 1 and two patients assigned to Group 2. This procedure will allow for a similar number of endorectal balloon patients (Group 1) and rectal spacer patients (Group 2), in the event that the planned sample size of 20 patients per group is not achieved.

12.3 Stratification Factors

Patients will not be risk-stratified.

12.4 Analysis of Primary and Secondary Endpoints

Primary Endpoint:

The primary endpoint of the acute grade I/II GU/GI toxicity proportions will be calculated in both groups and 95% confidence intervals will be estimated via binomial proportions. The chi-square test or Fisher's exact test will be used, as appropriate, to compare the toxicity proportions between the two arms. Ninety-five percent confidence intervals for the difference in the toxicity proportions between the two groups will be estimated via binomial proportions. The co-primary endpoint of rectal dose (Dmax, V35) will be compared between the two groups using the two-sample t-test/Wilcoxon rank-sum test or chi-square test/Fisher's exact test, as appropriate, for continuous and categorical formulations of rectal dose, respectively.

Secondary Endpoints:

Secondary endpoints will be compared between the two groups using the chi-square test or Fisher's exact test, as appropriate, for 1) EPIC bowel, urinary, and sexual domain outcomes, and 2) late GI/GU toxicity. Secondary endpoints will be compared between the two groups using the two-sample t-test or Wilcoxon rank-sum test, as appropriate, for 1) dose distribution and 2) CBC measurements.

The secondary endpoint of biochemical recurrence-free survival (BRFS) will be estimated using the Kaplan-Meier method, and 95% confidence intervals for the BRFS estimates will be calculated using Greenwood's formula. Median BRFS will also be estimated along with a 95% confidence interval. These calculations will be stratified by Group 1 vs. Group 2 and the log-rank test will be used to compare Kaplan-Meier BRFS between the two arms (for exploratory purposes only).

The frequency of subjects experiencing specific toxicities will be tabulated for each arm. Toxicities will be assessed and graded according to CTCAE v. 4.0 terminology. Exact 95% confidence intervals around the toxicity proportions will be calculated to assess the precision of the obtained estimates.

All p-values will be two-sided with statistical significance evaluated at the 0.05 alpha level. Ninety-five percent confidence intervals for all parameters will be calculated to assess the precision of the obtained estimates. All analyses will be performed in SAS Version 9.4 (SAS Institute, Inc., Cary, NC) and Stata Version 14.0 (StataCorp, College Station, TX).

12.5 Analysis of Correlative Endpoints

Microbiome

12.5.1 Sample size justification

A minimum of 25 patients will be required for this analysis. We calculated the minimum detectable difference based on single feature comparison between the subjects who will exhibit signs of mucosal injury (GI toxicity) versus those who will not. The feature could be the single taxon relative abundance or diversity index in describing microbiome distribution. If data are not normally distributed, we will normalize the data by either arcsine (i.e. for the relative abundance of each taxon or cluster) or log-normal. Based on the two-sided one sample paired t-test, to compare the microbiome change between before treatment and after treatment or the microbiome difference between affected versus non-affected patients (assuming rate of acute GI toxicity to be 70% grade 1, 5% grade 2)[37], the minimal detectable differences in term of σ at null with power=80% and α =0.05 is 0.58 σ.

12.5.2 Statistical analysis of microbiome and metagenome composition

Statistical analysis of microbiome and metagenome composition will be performed in the R statistical programming environment [38] using package *phyloseq* [39], which incorporates and builds upon community ecology packages such as ade4 and vegan and employs the flexible graphic

system ggplot2, to easily visualize complex data relationships. For 16S data, we will evaluate the adequacy of sequencing efforts using rarefaction plots. Alpha diversity index for each will be characterized through dominance, equitability, richness, evenness. The diversity metrics will be calculated at OTU and higher taxonomical levels to best characterize the community structure. We will test for associations of each of these alpha diversity metrics with the time relative to radiation exposure, using one-way ANOVA after even-sampling the observations to a depth cut-off maximizing the number of samples and depth. In addition, rank-abundance plots will be used to visualize differences in abundance of dominant taxa in the clinical and phenotypic groups. We will utilize skyline plots to visualize the patterns of community structure in terms of relative abundances in the collected samples between before and after the radiation treatment or between case and control samples. Similarly, for metagenomic data, skyline plots will be used to reveal functional compositions of the samples. Heat-maps will be plotted to visualize clustering patterns in the data.

Univariate analyses. To circumvent instabilities associated with rare species, which are difficult to detect uniformly in all samples, we will focus our primary univariate analyses only on highly abundant taxa, i.e. those present at 1% or more relative abundance across all specimens. This approach will also help to reduce multiple comparison-related Type I-error inflation, which will be formally controlled by false discovery rate (FDR) [40]. The differences in presence or absence of specific taxa and functional categories will be assessed by χ^2-test at all taxonomical levels. *Paired*-Sample Wilcoxon Signed Rank *Test* will be used to establish the differences in relative abundance of taxa between before and after the radiation treatment or between paired breastl samples. Depending on the dynamics observed, subsequent analyses may focus on less abundant taxa, and at higher depth, as indicated above.

Multivariate analyses. Multivariate association of the entire microbiome/metagenome with clinical and phenotypic factors will be examined with Principal Coordinate Analysis (PCoA) on Jensen-Shannon, weighted and unweighted Unifrac, and χ^2 (correspondence analysis) distances. Likewise, these distances will be used to build non-parametric multivariate ANOVA models (ADONIS) [41] to allow for simultaneous measurement of univariate and interaction effects of the clinico-phenotypic variables on the microbiome. Starting with the microbiota and metagenomic features significantly different in univariate analyses, we will use the dimension-reduction method, canonical correlation analysis (CCA) to identify the bacteria taxa which are related radiation treatment, according to benchmarked methodologies of our prior studies [42,43]. We will utilize a phylogenetic structure-constrained penalty function to impose phylogenetic relationships among bacteria on the model selection [44].

Longitudinal analysis: We will study the evolution of microbiome over time and how that evolution associated with the radiation treatment. The relative abundances at each taxonomical level will be first normalized by log-ratio transformation [45]. Then the transformed relative abundance of each individual taxa at multiple time points will be fitted by the linear mixed model along with the time effect and all subject-specific characteristics as the independent covariates. For the nonlinear trend, we will combine the nature splines with linear mixed model in the data analysis. The same model will be applied on the indices calculated in the ecology microbial analysis. For the joint analysis of more than one taxon. we will use MCMCglmm R

package to implement the multivariate generalized linear mixed model. This package gives a large flexibility in analyzing correlated multiple longitudinal response by allowing different types of covariance structure [46].

12.6 Reporting and Exclusions

12.6.1 Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first radiation treatment.

12.6.2 Evaluation of response. Disease control is monitored via PSA blood test for most routine cases.

13.0 Data and Safety Monitoring Plan (DSMP)

The WCMC Data and Safety Monitoring Committee (DSMC) is the central monitoring board for this study.

13.1 Monitoring plan

This study will be conducted in accordance with the guidelines in the 2001 NCI approved data Safety and Monitoring plan for the WCMC Cancer Institute Monitoring will occur on a yearly basis from the date the first patient is enrolled. Reports to the Data Safety and Monitoring Committee will include the following information: accruals, targets, responses, adverse events and evidence of reporting to appropriate review committees. The WCMC Data and Safety Monitoring Board (DSMB) will review the IRB approved protocol, the data and safety monitoring plan and any stopping guidelines during protocol initiation. During the course of the study, the DSMB will review cumulative study data twice a year to evaluate safety, efficacy, study conduct, and scientific validity and integrity of the trial. The WCMC DSMB may also convene as needed if stopping criteria are met or other safety issues arise that the Principal Investigator and/or IRB would like the WCMC DSMB to address.

13.2 Stopping rules

There are no stopping rules for this study.

14.0 REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* Jan 2016;66(1):7-30.
2. Network NCC. Prostate Cancer. 2016(01.2016).

3. Katz A, Kang J. Stereotactic body radiotherapy with or without external beam radiation as treatment for organ confined high-risk prostate carcinoma: a six year study. *Radiat Oncol.* 2014;9:1.
4. Katz A, Formenti SC, Kang J. Predicting Biochemical Disease-Free Survival After Prostate Stereotactic Body Radiotherapy: Risk-Stratification and Patterns of Failure. *Under review.* 2016.
5. Oermann EK, Slack RS, Hanscom HN, et al. A pilot study of intensity modulated radiation therapy with hypofractionated stereotactic body radiation therapy (SBRT) boost in the treatment of intermediate- to high-risk prostate cancer. *Technology in cancer research & treatment.* Oct 2010;9(5):453-462.
6. King CR, Freeman D, Kaplan I, et al. Stereotactic body radiotherapy for localized prostate cancer: pooled analysis from a multi-institutional consortium of prospective phase II trials. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology.* Nov 2013;109(2):217-221.
7. Katz AJ, Kang J. Stereotactic body radiotherapy as treatment for organ confined low- and intermediate-risk prostate carcinoma, a 7-year study. *Frontiers in oncology.* 2014;4:240.
8. D'Amico AV, Manola J, Loffredo M, et al. A practical method to achieve prostate gland immobilization and target verification for daily treatment. *Int J Radiat Oncol Biol Phys.* Dec 1 2001;51(5):1431-1436.
9. Bastasch MD, Teh BS, Mai WY, McGary JE, Grant WH, 3rd, Butler EB. Tolerance of endorectal balloon in 396 patients treated with intensity-modulated radiation therapy (IMRT) for prostate cancer. *American journal of clinical oncology.* Feb 2006;29(1):8-11.
10. Teh BS, Dong L, McGary JE, Mai WY, Grant W, 3rd, Butler EB. Rectal wall sparing by dosimetric effect of rectal balloon used during intensity-modulated radiation therapy (IMRT) for prostate cancer. *Medical dosimetry : official journal of the American Association of Medical Dosimetrists.* Spring 2005;30(1):25-30.
11. Wong AT, Schreiber D, Agarwal M, Polubarov A, Schwartz D. Impact of the use of an endorectal balloon on rectal dosimetry during stereotactic body radiation therapy for localized prostate cancer. *Pract Radiat Oncol.* Nov 9 2015.
12. Patel RR, Orton N, Tome WA, Chappell R, Ritter MA. Rectal dose sparing with a balloon catheter and ultrasound localization in conformal radiation therapy for prostate cancer. *Radiother Oncol.* Jun 2003;67(3):285-294.
13. van Lin EN, Hoffmann AL, van Kollenburg P, Leer JW, Visser AG. Rectal wall sparing effect of three different endorectal balloons in 3D conformal and IMRT prostate radiotherapy. *Int J Radiat Oncol Biol Phys.* Oct 1 2005;63(2):565-576.
14. Wachter S, Gerstner N, Dorner D, et al. The influence of a rectal balloon tube as internal immobilization device on variations of volumes and dose-volume histograms during treatment course of conformal radiotherapy for prostate cancer. *Int J Radiat Oncol Biol Phys.* Jan 1 2002;52(1):91-100.
15. Jones BL, Gan G, Diot Q, Kavanagh B, Timmerman RD, Miften M. Dosimetric and deformation effects of image-guided interventions during stereotactic body radiation therapy of the prostate using an endorectal balloon. *Med Phys.* Jun 2012;39(6):3080-3088.
16. Mariados N, Sylvester J, Shah D, et al. Hydrogel Spacer Prospective Multicenter Randomized Controlled Pivotal Trial: Dosimetric and Clinical Effects of Perirectal

Spacer Application in Men Undergoing Prostate Image Guided Intensity Modulated Radiation Therapy. *Int J Radiat Oncol Biol Phys.* Aug 1 2015;92(5):971-977.

17. Prada PJ, Jimenez I, Gonzalez-Suarez H, Fernandez J, Cuervo-Arango C, Mendez L. High-dose-rate interstitial brachytherapy as monotherapy in one fraction and transperineal hyaluronic acid injection into the perirectal fat for the treatment of favorable stage prostate cancer: treatment description and preliminary results. *Brachytherapy.* Mar-Apr 2012;11(2):105-110.

18. Strom TJ, Wilder RB, Fernandez DC, et al. A dosimetric study of polyethylene glycol hydrogel in 200 prostate cancer patients treated with high-dose rate brachytherapy+/-intensity modulated radiation therapy. *Radiother Oncol.* Apr 2014;111(1):126-131.

19. Beydoun N, Bucci JA, Chin YS, Malouf D, Enari E, Painter SD. First report of transperineal polyethylene glycol hydrogel spacer use to curtail rectal radiation dose after permanent iodine-125 prostate brachytherapy. *Brachytherapy.* Jul-Aug 2013;12(4):368-374.

20. Chapet O, Decullier E, Bin S, et al. Prostate hypofractionated radiation therapy with injection of hyaluronic acid: acute toxicities in a phase 2 study. *Int J Radiat Oncol Biol Phys.* Mar 15 2015;91(4):730-736.

21. Skwarchuk MW, Jackson A, Zelefsky MJ, et al. Late rectal toxicity after conformal radiotherapy of prostate cancer (I): multivariate analysis and dose-response. *Int J Radiat Oncol Biol Phys.* Apr 1 2000;47(1):103-113.

22. Storey MR, Pollack A, Zagars G, Smith L, Antolak J, Rosen I. Complications from radiotherapy dose escalation in prostate cancer: preliminary results of a randomized trial. *Int J Radiat Oncol Biol Phys.* Oct 1 2000;48(3):635-642.

23. Snyder KM, Stock RG, Hong SM, Lo YC, Stone NN. Defining the risk of developing grade 2 proctitis following 125I prostate brachytherapy using a rectal dose-volume histogram analysis. *Int J Radiat Oncol Biol Phys.* Jun 1 2001;50(2):335-341.

24. Tanyi JA, He T, Summers PA, et al. Assessment of planning target volume margins for intensity-modulated radiotherapy of the prostate gland: role of daily inter- and intrafraction motion. *Int J Radiat Oncol Biol Phys.* Dec 1 2010;78(5):1579-1585.

25. Sumila M, Mack A, Schneider U, Storelli F, Curschmann J, Gruber G. Long-term intra-fractional motion of the prostate using hydrogel spacer during Cyberknife(R) treatment for prostate cancer--a case report. *Radiat Oncol.* 2014;9:186.

26. Ruggieri R, Naccarato S, Stavrev P, et al. Volumetric-modulated arc stereotactic body radiotherapy for prostate cancer: dosimetric impact of an increased near-maximum target dose and of a rectal spacer. *Br J Radiol.* Oct 2015;88(1054):20140736.

27. Pinkawa M, Schubert C, Escobar-Corral N, Holy R, Eble MJ. Application of a hydrogel spacer for postoperative salvage radiotherapy of prostate cancer. *Strahlenther Onkol.* Apr 2015;191(4):375-379.

28. Villa A, Sonis ST. Mucositis: pathobiology and management. *Curr Opin Oncol.* May 2015;27(3):159-164.

29. Sonis ST. The pathobiology of mucositis. *Nat Rev Cancer.* Apr 2004;4(4):277-284.

30. Blijlevens NM, Donnelly JP, DePauw BE. Inflammatory response to mucosal barrier injury after myeloablative therapy in allogeneic stem cell transplant recipients. *Bone Marrow Transplant.* Oct 2005;36(8):703-707.

31. van Vliet MJ, Harmsen HJ, de Bont ES, Tissing WJ. The role of intestinal microbiota in

the development and severity of chemotherapy-induced mucositis. *PLoS Pathog.* May 2010;6(5):e1000879.

32. Manichanh C, Varela E, Martinez C, et al. The gut microbiota predispose to the pathophysiology of acute postradiotherapy diarrhea. *Am J Gastroenterol.* Jul 2008;103(7):1754-1761.

33. de la Torre JR, Christianson LM, Beja O, et al. Proteorhodopsin genes are distributed among divergent marine bacterial taxa. *Proceedings of the National Academy of Sciences of the United States of America.* Oct 28 2003;100(22):12830-12835.

34. Dethlefsen L, Eckburg PB, Bik EM, Relman DA. Assembly of the human intestinal microbiota. *Trends in ecology & evolution.* Sep 2006;21(9):517-523.

35. von Mering C, Hugenholtz P, Raes J, et al. Quantitative phylogenetic assessment of microbial communities in diverse environments. *Science.* Feb 23 2007;315(5815):1126-1130.

36. Nelson KE, Methe BA, Kowalchuk GA. Microbial environmental genomics. *Microbial ecology.* Apr 2007;53(3):367-368.

37. Katz A, Ferrer M, Suarez JF, Multicentric Spanish Group of Clinically Localized Prostate C. Comparison of quality of life after stereotactic body radiotherapy and surgery for early-stage prostate cancer. *Radiat Oncol.* 2012;7:194.

38. Team" RDC. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2012.

39. McMurdie PJ, Holmes S. Phyloseq: a bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. Pacific Symposium on Biocomputing. Pacific Symposium on Biocomputing. *http://bioconductor.org/packages/devel/bioc/vignettes/phyloseq/inst/doc/phyloseq_analysis.pdf*. 2012.

40. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *J Roy Stat Soc B Met.* 1995;57(1):289-300.

41. Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecology.* 2001;26(1):32-46.

42. Statnikov A, Alekseyenko AV, Li ZG, et al. Microbiomic Signatures of Psoriasis: Feasibility and Methodology Comparison. *Sci Rep-Uk.* Sep 10 2013;3.

43. Statnikov A, Henaff M, Narendra V, et al. A comprehensive evaluation of multicategory classification methods for microbiomic data. *Microbiome.* 2013;1(1):11.

44. Chen J, Bushman FD, Lewis JD, Wu GD, Li H. Structure-constrained sparse canonical correlation analysis with an application to microbiome data analysis. *Biostatistics.* Apr 2013;14(2):244-258.

45. Aitchison J. *The statistical analysis of compositional data*: Chapman & Hall, Ltd.; 1986.

46. Hadeld J. MCMC methods for Multi-response Generalised Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software.* 2010;33(2):1-22.