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Title: A Phase I Trial of Highly Conformal, Hypofractionated, Focally Dose Escalated Post-Prostatectomy Radiotherapy

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Radiation

PRÉCIS

Background:

Prostate cancer that recurs after prostatectomy (rising PSA) with no evidence of metastatic disease is often treated with radiation to the entire prostate bed to a dose of 66-72 Gy over 6-7 weeks. This treatment can provide PSA control in approximately 75% of patients, but may have associated genitourinary and gastrointestinal toxicity due to irradiation of the rectum, small bowel, and bladder. Imaging of prostate cancer has improved to the extent that recurrent disease is often identified in the prostate bed or in other pelvic sites. The current standard is to irradiate the entire prostate bed to the total dose. This trial will test the tolerability of accelerated treatment designed to yield a similar rate of late toxicity. In addition, in patients with visible tumor, it will test the feasibility of delivering a lower dose to the prostate bed and an integrated boost (simultaneous) to the visible tumor to allow a higher dose to visible tumor than can be delivered with standard approaches.

Objective:

- Define the maximum tolerated dose (MTD) hypofractionation of image guided, focally dose escalated post-prostatectomy radiation.

Eligibility:

- PSA recurrence after prostatectomy or indications for adjuvant radiation after prostatectomy.
- No evidence of distant metastases of prostate cancer (pelvic lymph nodes are allowed).
- Age ≥18 years old
- ECOG performance status ≤1

Design:

This is a Phase I trial of hypofractionated, focal dose escalation with reduced dose prostate bed irradiation using image and pathologic guidance. The prostate bed will be treated with hypofractionated radiation and areas in the prostate bed or pelvis shown to have tumor on biopsy or with advanced imaging studies will be treated with an integrated boost to visible tumor. The treatment duration will be decreased sequentially in three Dose Level groups. Quality of life and functional outcomes such as urine, bowel, and erectile function will be assessed with questionnaires. A maximum of 48 patients will be enrolled.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Define the maximum tolerated dose (MTD) hypofractionated of image guided, focally dose escalated post-prostatectomy radiation.

1.1.2 Secondary Objectives

- To evaluate the safety and tolerability of the image guided, hypofractionated, focally dose escalated post-prostatectomy radiation.
- Estimate the rate of PSA control (biochemical progression free survival (bPFS) at 1 and 2 years after treatment with image guided, hypofractionated, focally dose escalated post-prostatectomy radiation).
- Describe the effects of image-guided, focally dose escalated prostate bed radiation on patient reported outcomes (SHIM, AUA Symptom Index, EPIC-26).

1.1.3 Exploratory Objectives

- Evaluate decision regret at the time of enrollment and at the end of follow up relating to the primary treatment for prostate cancer to determine if successful salvage mitigates decision regret.
- Explore candidate biomarkers of radiation toxicity in blood and urine of patients receiving image-guided, hypofractionated, focally dose escalated post-prostatectomy radiation.
- Explore MRI imaging parameters of erectile tissues in regard to testosterone and erectile dysfunction
- To compare dosimetry of standard compared to biological optimization algorithms for treatment planning of post-prostatectomy radiation.
- Explore changes in circulating T-cell subsets, circulating T-cell polarization, and circulating myeloid lineages during and after treatment with radiation.
- Evaluate the feasibility of a patient specific circulating DNA copy number analysis for predicting biochemical recurrence
- Explore changes in circulating exosome cargo before, during, and after a course of radiotherapy.

1.2 BACKGROUND AND RATIONALE

1.2.1 Post prostatectomy radiation

Prostate cancer is one of the most common malignancies in men in the United States, with an estimated 161,360 new cases and 26,730 deaths in 2017 [1]. Localized prostate cancer is often treated with radiotherapy or prostatectomy. Both treatments can be exceptionally effective for patients with low grade cancers or low pre-treatment PSA values. In patients with intermediate or high grade tumors (Gleason 7-10), histologic variants, locally advanced disease (disease that has spread beyond the prostate capsule or into the seminal vesicles), or a high pretreatment PSA, the rate of recurrence after potentially curative treatment, such as radiation or surgery, can be

much higher. Long term follow up of patients treated with prostatectomy have yielded 10 year recurrence free rates of 68% [2]. Subsets of patients at highest risk of failure can benefit from post-operative radiation to the prostate bed, even in the absence of a detectable PSA. When given in the absence of a PSA recurrence, the treatment is termed "adjuvant radiation." Adjuvant radiation, given for high risk features such as seminal vesicle invasion, extra prostatic spread, or positive margin, has been shown to improve progression free survival and improve the overall survival of prostate cancer patients when compared to observation [3-6].

One concern with the use of adjuvant radiation is that some patients with high risk features will never develop PSA recurrence. Therefore, the benefit of post-prostatectomy radiation may be limited to those destined for eventual PSA recurrence, while many other patients receiving the treatment would never benefit. Thus, many patients with indications for adjuvant radiation after prostatectomy are instead monitored carefully after surgery for evidence of PSA recurrence. If the PSA rises after a prostatectomy and there is no evidence of extra-pelvic metastatic disease, patients are treated with radiotherapy to the prostate bed with curative intent, also known as "salvage" radiotherapy. Treatment with post-operative radiotherapy in the setting of PSA recurrence after radiation yields a 5 year progression free survival of 62-80%[7]. Comparisons of adjuvant and salvage radiation are currently ongoing [8], however both are considered reasonable options as per the current standard of care.

The volumes targeted by irradiation in the post-prostatectomy setting (adjuvant or salvage) are relatively standard and have been arrived at by consensus [9]. In general, the remaining seminal vesicles, if any, and the bed of the prostate are included. In some cases, a portion of the pelvic lymph nodes are included. Most commonly, this region receives a total dose of 66 to 72 Gy in 1.8- 2 Gy fractions, given daily Monday through Friday, over 6-7 weeks. Although these treatments are delivered on an outpatient basis, they are a substantial investment of time and may require time away from work and incur transportation costs.

In general, the use of adjuvant and salvage radiation is well tolerated, however a subset of patients will develop acute (during or within 90 days of radiation) or late (more than 90 days after completion of radiation) toxicity. Two recently published randomized trials provide estimates of toxicity with contemporary irradiation techniques [7, 10]. Grade 2 or higher acute genitourinary toxicity occurs in approximately 14% of patients, whereas Grade 2 or higher acute gastrointestinal toxicity occurs in 21% of patients (acute Grade 3 or higher GU and GI, 2% and 0.5% respectively). Grade 2 or higher late genitourinary toxicity occurs in approximately 25% of patients, whereas Grade 2 or higher late gastrointestinal toxicity occurs in 18% of patients (late Grade 3 or higher GU and GI, 7% and 2% respectively).

Radiation is often combined with androgen deprivation therapy (ADT) in patients with prostate cancer treated with salvage radiation after prostatectomy (detectable PSA). Two recently reported randomized trials of salvage radiation after prostatectomy delivered with or without ADT have shown a benefit in progression free survival or overall survival [7, 10]. These trials demonstrated no increase in bowel or bladder toxicity with the addition of ADT to radiation compared to radiation alone. Based on these results, the use of ADT with radiation is considered appropriate in some, but not all, patients receiving salvage radiation. The use of ADT is dependent of specific tumor and patient characteristics, including age, comorbidity, PSA at the time of radiation, Gleason score, margin status, presence of extra prostatic extension, and patient willingness. We will allow patients to receive ADT on this trial if deemed clinically appropriate,

as the bulk of data suggests that it has no impact on the toxicities we have defined as DLTs in this trial.

1.2.2 Hypofractionation in prostate cancer

Hypofractionation in radiotherapy is the use of fractional doses larger than the "standard" 1.8 Gy – 2 Gy fractions size, with the total dose delivered in a more rapid time frame. Hypofractionated approaches have long been used in the palliative setting to rapidly complete treatment in patients with limited life expectancies. The capacity to utilize modern advanced radiation techniques, such as intensity modulated radiation therapy and stereotactic body radiation therapy, to maximally treat tumor while minimizing the exposure of normal tissues to high doses has led to increased interest in hypofractionated approaches.

For certain cancers, the growth kinetics and radiation response further support the use of hypofractionated approaches over "standard" 2Gy per day fractionation approaches. A substantial body of evidence suggests that the α/β ratio for prostate cancer is lower than for many cancers, and even for many late responding normal tissues. The α/β ratio is a descriptive value based on the curvature of a cell survival curve [11]. The alpha beta ratio is the dose at which radiation cell killing from linear and quadratic components are equal. In general, early responding tissues, such as mucosa, bone marrow, and skin, have high α/β ratios (on the order of 10) [11]. Late responding tissues, such as brain, spinal cord, muscle, and dermis have very low α/β ratios (on the order of 3) [11]. Tumors can have a range of alpha beta ratios, however certain histologies, such as prostate cancer, melanoma, and sarcoma, have been described to have very low α/β ratios. The clinical implication of this information is that tumors with low α/β ratios, such as prostate cancer, should respond favorably to large dose per fraction (hypofractionated) approaches.

Although much of this work in defining alpha beta ratios was initiated in vitro, studies of patients treated with radiotherapy have been used to confirm that the α/β ratio of prostate cancer is indeed low [12]. These findings have been used as the rationale for developing hypofractionated regimens for the treatment of patients with previously untreated prostate cancer [13]. Several randomized trials of hypofractionated radiotherapy compared to standard fractionation in patients with intact prostates have suggested that this approach is effective and well tolerated [14-16].
Mathematical calculations can be used to determine isoeffect levels (biologically equivalent dose) using the formula $BEDx=TD(1+x/[\alpha/\beta])$, where x is the fraction size, TD is the total dose delivered, and α/β is the ratio of the tissue of interest. Few, if any, normal tissues have an α/β ratio less than 3. Thus, by using the α/β ratio of 3 to drive choice of an appropriate hypofractionated regimen, tissues with higher α/β ratios may actually benefit from hypofractionation as the BED of these fractionation regimens may be biologically "less dose" when considering a α/β higher than 3. For example, delivering 68.4 Gy in 2 Gy fractions is equivalent to delivering 56.4 Gy in 2.82 Gy fractions if using an α/β of 3(prostate cancer, late responding normal tissues). The similar regimen delivers a dose biologically equivalent to 60.25 Gy in 2 Gy fractions (α/β ratio of 10, early responding tissues). Thus, the more sensitive normal tissues receive a reduced dose biologically while the tumor receives a biologically equivalent dose.

There is substantial interest in the use of hypofractionated radiotherapy for prostate cancer, largely because of the prevailing thought that prostate cancer exhibits a low α/β ratio. In addition, hypofractionated therapies are of increasing interest given enhanced conformality of radiation

treatments and enhanced convenience for patients. The vast majority of data evaluating outcomes of hypofractionated radiotherapy in the treatment of prostate cancer has been developed in the setting of an intact prostate (no prior surgery). The doses used in the setting of radiotherapy are substantially higher than those used in the post-prostatectomy setting (79-82 Gy compared to 66-72 Gy). Additionally, the volumes treated in the intact prostate setting are generally smaller than those treated in the post-prostatectomy setting. Regardless, these studies do provide information on the efficacy and tolerability of hypofractionated radiotherapy in a similar region of the body. In many cases, these regimens, although hypofractionated compared to the current standard, were substantially less hypofractionated than the regimen proposed herein.

A recent multi-center randomized trial (PROFIT) evaluated the efficacy of hypofractionated radiation in patients with intermediate risk localized prostate cancer in which 1,206 patients were randomized to conventional dosing (78 Gy in 2 Gy fractions) versus hypofractionated radiation (60 Gy in 3 Gy fractions) [17]. At a median follow-up of 6 years, the biochemical disease free survival was 85% in both arms (the study endpoint was non-inferiority), with no significant differences in late > Grade 3 genitourinary or gastrointestinal toxicity. Similarly, RTOG 0415 enrolled 1,115 men with low to intermediate risk prostate cancer to 73.8 Gy in conventional fractionation or 70 Gy in 2.5 Gy fractions. At a median follow-up of 5.8 years, there were more grade 2 toxicities in the hypofractionation arm, but grade 3 toxicities were similar. In this trial, the moderate hypofractionation regimen was also found to be non-inferior [18]. In the CHHiP trial, 3,216 men with intermediate risk prostate cancer were randomized to 74 Gy in conventional fractionation compared to 57-60Gy in 3 Gy fractions [14]. At 62 months of follow up, the hypofractionated regimen was not inferior. In this trial, there were more acute toxicities in the hypofractionated group and more late toxicity in the conventionally fractionated group. The HYPRO trial assessed 64.6 Gy in 3.4 Gy fractions (3 times per week) compared to 78 Gy standard fractionation. Other trials assessing superiority of hypofractionated radiotherapy versus conventionally fractionated radiotherapy in the setting of intact prostate have not found evidence of superiority [19-21]. As these dosing regimens are calculated for estimated equivalence of tumor control, these are findings are not particularly surprising.

In the post-prostatectomy setting, NRG Oncology has recently activated a trial of hypofractionated radiotherapy post-prostatectomy (NRG GU003). In this trial, 282 patients will be randomized to hypofractionated radiotherapy or conventionally fractionated radiotherapy (66.6 Gy in 1.8 Gy fractions versus 62.5 Gy in 2.5 Gy fractions). In the RTOG trial, the hypofractionated arm also includes a dose escalation component (the entire prostate bed receives the hypofractionated dose which is biologically equivalent to an escalated dose). Supporting studies for this trial included a series from the University of Wisconsin, which evaluated 65 Gy in 2.5 Gy fractions to the prostate bed with a resulting 4 year progression free survival of 67% at a median follow up of 34 months [22]. In this trial, late toxicity was highly favorable compared to historical series.

This trial is substantially different from published series in that it is not “moderate” hypofractionation. This approach is substantially more hypofractionated compared to regimens previously investigated in this context and much more in line with modern hypofractionated SBRT approaches that have been used for radiation of the intact prostate. Second, this trial includes an escalation component in which the imaged tumor is escalated while dose to the remaining prostate bed is reduced to a dose that is biologically equivalent to a “microscopic”

radiotherapy dose. Finally, the trial attempts to integrate novel quality of life, biomarker, and correlative endpoints to further explore the effects of this rapid treatment.

1.2.3 Quality of life after salvage radiation

Altering the dose per fraction given during radiation treatments not only impacts malignant cells, but also surrounding normal tissues. As the dose per fraction received by these tissues changes, so also may the rate of acute and late toxicity. Therefore, assessment of acute and late toxicity and resultant quality of life effects is an important component of outcomes assessment in altered fractionation radiation regimes. Validated measures such as the EPIC-26, AUA, and SHIM are key in assessing both objective and subjective patient reported outcomes post-treatment. They focus on bowel and urinary toxicity, as well as sexual and erectile function, which can all be negatively affected by treatments for prostate cancer, in both the upfront and salvage settings [23, 24].

While altered fractionation may have a potential benefit in tumor cell kill, novel fractionation modalities must also address patient quality of life particularly with adjuvant and salvage radiation therapy post-prostatectomy [25, 26]. Decision regret after prostate cancer is a recognized phenomenon occurring in 10-20% of patients after upfront treatment [27-30]. Decision regret has been associated with time since treatment, toxicities, and patient demographics [29-31]. Increased levels of regret have been associated with bowel or bladder toxicity from, but independent of, treatment modality [24, 27, 28], and may be more common in men who experience PSA recurrence or metastatic disease [31]. Despite these efforts, a comprehensive schema of factors involved in decision regret has not yet been established. It is important to explore factors such as mood and patients' views on their disease in order to better understand the components of decision regret, and how they can best be mitigated. Uniquely in this research, we will address patients who have options for curative intent salvage treatments, rates of decision regret post-prostatectomy, and if subsequent regret can be mitigated by effective salvage therapies.

The questionnaires used in this trial are:

- The Expanded Prostate Cancer Index Composite (EPIC-26) is a prostate cancer health-related quality of life (HRQOL) patient self-administered instrument that measures urinary, bowel, sexual, and hormonal symptoms related to radiotherapy and hormonal therapy. The EPIC-26 was developed from the 50-item EPIC questionnaire and was validated using question responses from 252 subjects who had undergone brachytherapy, external beam radiotherapy, or prostatectomy for prostate cancer. A high correlation was observed between the EPIC-50 and EPIC-26 versions for the urinary incontinence, urinary irritation/obstruction, bowel, sexual, and vitality/hormonal domain scores (all r >/=0.96). The correlations between the different domains were low, confirming that EPIC-26 retained the ability to discern the distinct HRQOL domains. The internal consistency and test-retest reliability for EPIC-26 (Cronbach's alpha >/=0.70 and r >/=0.69, respectively for all HRQOL domains) supported its validity. EPIC-26 has been used in multiple RTOG protocols and phase II publications for hypofractionated radiotherapy to the prostate.
- Sexual Health Inventory for Men (SHIM) is a prostate specific questionnaire for assessment of erectile dysfunction. Initially derived from the IIEF, a shortened version to six questions the IIEF-6 or SHIM was created for increased usability in the clinic.

- American Urologic Association Symptom Index Score (AUA-SI) has been established as a measure of radiation morbidity in patients treated for prostate cancer with respect to urinary function and is used routinely as the basis for treatment decisions. Moderate agreement was observed between EPIC domains and the American Urological Association Symptom Index (AUA-SI), providing criterion validity without excessive overlap.
- PROMIS – Depression SF4a: The PROMIS Depression item bank assesses self-reported negative mood (sadness, guilt), views of self (self-criticism, worthlessness), and social cognition (loneliness, interpersonal alienation), as well as decreased positive affect and engagement (loss of interest, meaning, and purpose). The depression short forms are universal rather than disease-specific. All assess depression over the past seven days. PROMIS instruments are scored using item-level calibrations.
- PROMIS – Anxiety SF4a: The PROMIS Anxiety item banks assess self-reported fear (fearfulness, panic), anxious misery (worry, dread), hyperarousal (tension, nervousness, restlessness), and somatic symptoms related to arousal (racing heart, dizziness). Anxiety is best differentiated by symptoms that reflect autonomic arousal and experience of threat. Only one behavioral avoidance item is included in the adult item bank; therefore, behavioral fear avoidance is not fully evaluated. The anxiety measures are universal rather than disease-specific. All assess anxiety over the past seven days.
- PROMIS – Psychosocial Impact Positive SF 4a assess positive psychosocial (emotional and social) outcomes of illness, previously conceptualized in various ways including post-traumatic growth, benefit-finding, and meaning making. Positive Illness Impact refers to positive psychosocial outcomes of illness that can occur as a result of confrontation with one's mortality, such as greater life appreciation, interpersonal relationships and personal resources.
- Decision Regret Scale (DRS) is a 5-item scale measuring distress or remorse after a health case decision. It has been psychometrically validated in the prostate cancer population. The score correlated with satisfaction with the decision (r=-0.40 to -0.60), decisional conflict (r=0.31 to 0.52), and overall rated quality of life (r=-0.25 to -0.27).

1.2.4 Biological versus standard optimization algorithms

Radiation treatment planning for intensity modulated radiation therapy typically uses inverse planning algorithms, whereby the physician defines dose limits to organs at risk of injury and defines the dose goals to the target. The computer then iteratively optimizes the radiation delivery such that it attempts to reach the dose goal to the target while respecting the organ at risk (normal tissue) dose constraints. A number of dose limits have been published for normal tissues and, in general, these are often adapted from those included on randomized trials or from the QUANTEC project [32]. Biological optimization uses biological modeling to incorporate local tumor control probability data (TCP) and normal tissue complication probability data (NTCP) into the planning process. This approach requires integration of TCP and NTCP data into the treatment planning process. Although patients will be treated using standard planning techniques, we will explore the capacity of this process to alter radiation treatment planning retrospectively from the imaging datasets obtained on this trial.

1.2.5 Summary

The goal of this study is to evaluate a new regimen that would reduce the duration of treatment compared to the current standard. For patients with no evidence of tumor on clinical evaluation (imaging or biopsy as clinically indicated) this would be accomplished by increasing the fraction size to the prostate bed to allow for more rapid treatment. Because the dose is delivered more rapidly, the total dose would be adjusted for biological equivalence to tumor when compared to standard fractionation. Thus, an equivalent biological dose would be delivered to the tumor bed more rapidly than the current standard. We anticipate that this approach will be tolerable as newer technologies have provided the capacity to enhance the conformality of dose around the target. In addition, daily imaging guidance allows increased confidence that normal tissue is maximally spared and tumor tissue is included. This approach has begun to be explored with conservative increases in fraction size (2.2 - 2.5 Gy) and appears safe [33-38]. In patients with no visible tumor, our study aims to accelerate using the most modern techniques, with daily image guidance, and with consideration of not only toxicity, but Quality of Life (QoL).

In patients with tumor identified on imaging studies or on clinical biopsy, we aim to reduce the dose delivered to the prostate bed to a level considered adequate of sterilizing microscopic disease and escalate dose to the areas of known recurrence. In general, the site of local recurrence after prostatectomy has historically not been known after prostatectomy based on limitations with imaging in the post-prostatectomy setting. Occasionally, an area would be palpated in the bed on digital rectal examination, but often not until the PSA was higher than what is traditionally used as a cut off for delivering radiation treatments. Historically, MRI was not performed and CT imaging gave poor soft tissue contrast in this anatomic location. More recently, it has become clear that recurrences can often be identified with high quality MR imaging using an endorectal coil or advanced imaging techniques, and in many cases these areas are biopsied for confirmation [39, 40]. Because the site of recurrence is known, this provides the opportunity in this patient subset to escalate dose to the recurrent disease while reducing the dose to the prostate bed.

Collectively, these approaches are evaluating the tolerability of more rapidly delivering radiation and reducing normal tissue exposure through the use of highly conformal radiation treatments.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically or cytologically confirmed adenocarcinoma of the prostate.

2.1.1.2 Indications for post-prostatectomy radiation exist:

- Disease progression (detectable PSA on two measurements obtained at least one month apart) or
- indications for adjuvant radiation exist (if undetectable PSA): pathologic T3, T4, N+ disease or positive margins (within 1 year of prostatectomy).

2.1.1.3 Age ≥18 years.

2.1.1.4 ECOG performance status ≤1 (Karnofsky ≥60%, see **Appendix A**)

2.1.1.5 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.1.6 Radiation is teratogenic; thus, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and up to 120 days after the last radiation. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.7 HIV positive patients are included if CD4+ T-cell count > 200 cells/uL; on stable antiretroviral therapy for > 1 year with HIV viral load <200 copies/mL, and no history of opportunistic infections in > 1 year.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents concurrently.

2.1.2.2 Documented metastases of prostate cancer outside of the pelvis (pelvic lymph nodes are allowed only if within the prostate bed region).

2.1.2.3 History of radiation that would overlap with the intended treatment to the prostate bed.

2.1.2.4 Known contraindications to radiation such as inflammatory bowel disease, active systemic lupus or scleroderma, or radiation hypersensitivity syndrome (Ataxia Telangiectasia or Fanconi's Anemia)

2.1.2.5 Subjects with any coexisting medical or psychiatric condition which, in the opinion of the Investigator likely to interfere with study procedures and/or results.

2.1.2.6 Medically indicated use of known radiosensitizing drugs (such as protease inhibitors)

2.1.3 Recruitment Strategies

Participants will be identified from Dr. Citrin's referral base, largely via referrals from the Urologic Oncology Branch, via the multi-disciplinary clinic, and via direct outside referrals to Dr. Citrin's clinic. Dr. Citrin has developed an excellent professional relationship with numerous referring physicians in the surrounding area who refer patients.

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

2.2 SCREENING EVALUATION

Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols). Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Within 4 weeks prior to enrollment unless otherwise noted below:

- Physical Evaluation: Complete medical history and physical examination, including vital signs and ECOG performance status.
- Laboratory Evaluations

- HIV Viral serology and HIV viral load
- CD4+ T-cell count for HIV positive patients
- PSA

- Radiographic Evaluations (within 4 months prior to enrollment):
  - multiparametric MRI (mpMRI) of the prostate bed
  - CT of the abdomen and pelvis (with oral and IV contrast) may also be obtained for evaluation of eligibility, particularly if MRI is not felt to adequately have staged the pelvic and abdominal lymph nodes
  - whole body bone scan or 18F NaF PET if felt to be required by the clinician; this imaging is not required if MRI and/or CT scans adequately staged the pelvic and abdominal lymph nodes, or if other standard scans were performed (e.g., 18F-DCFPyL) that confirm the disease status

- Histological or cytological pathology documentation, confirming adenocarcinoma of the prostate and for determination of whether indication for adjuvant radiation exists.
- Collection of medical records pertaining to medical history as well as testing and procedures for the diagnosis under study (e.g., imaging results including 18F-DCFPyL PET/CT)

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.4 TREATMENT ASSIGNMENT PROCEDURES

Cohorts

Number	Name	Description
1	Recurrent tumor visible	Recurrent tumor visible
2	Recurrent tumor NOT visible	Recurrent tumor NOT visible

Arms

Number	Name	Description
Arm 1	Prostate bed with integrated boost	Radiation will be delivered to an escalated dose to areas of recurrent prostate cancer identified on imaging and a reduced dose will be delivered to the entire prostate bed.
Arm 2	Prostate bed irradiation only	Radiation will be delivered to the prostate bed only

Arm Assignment

Patients in Cohort 1 will be directly assigned to Arm 1. Patients in Cohort 2 will be directly assigned to Arm 2.

2.5 BASELINE EVALUATION

Tests done at screening do not need to be repeated on baseline if performed in designated time frame prior to start of treatment:

Within 2 weeks prior to treatment initiation:

- Clinical assessment with attention to any new medications or interventions since screening history and physical examination including vital signs and ECOG performance status.
- Hematological profile: CBC with differential and platelet count,
- Lymphocyte phenotype TBNK
- Biochemical profile: electrolytes, creatinine, BUN, alkaline phosphatase, ALT, AST, total and direct bilirubin,
- PSA, testosterone,
- Questionnaires: Expanded Prostate Cancer Index Composite (EPIC-26), SHIM, AUA-SI, PROMIS Depression SF4a, PROMIS Anxiety SF4a, PROMIS Psychological Impact Positive SF 4a, Decision Regret Scale. For English speaking patients only.
- Collection of blood and urine for correlative assays (see section **5.1** for further details).
- If available, tissue blocks or sections from prostatectomy or biopsies will be requested

Within 4 months prior to treatment:

- Radiographic evaluation: Multiparametric MRI of the prostate bed.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

There will be two Arms in this trial. Arm 1 includes patients with rising PSA after prostatectomy for whom recurrent tumor has been visualized in the pelvis, providing a target for dose escalation. Arm 2 includes patients with rising PSA with no visualized tumor on imaging or patients with indications for adjuvant radiation after prostatectomy. Patients in whom MRI is contraindicated may enroll on Arm 2 if no evidence of disease is noted on other imaging modalities.

In Arm 1 radiation will be delivered to an escalated dose to areas of recurrent prostate cancer identified on imaging and a reduced dose will be delivered to the entire prostate bed.

In Arm 2 radiation will be delivered to the prostate bed only in patients for whom no recurrent tumor is visualized on imaging or if MRI imaging was contraindicated.

On Dose Level 1 treatments will be delivered in 20 fractions (approximately 4 weeks), on Dose Level 2 - in 15 fractions (approximately 3 weeks), on Dose Level 3 - in 10 fractions (approximately 2 weeks).

Treatment will be administered in daily fractions Monday through Friday except in the case of machine malfunction or federal holiday.

Daily image guidance with cone beam CT will be preferred. kV/kV imaging is allowed if the Cone beam CT imager is unavailable. An additional post-treatment cone beam CT after each fraction may be obtained to assess intrafraction motion.

Table 1 Fraction Dose Escalation Schedule

	Cohort 1, Arm1: Recurrent tumor visible, prostate bed with integrated boost		**Cohort 2, Arm 2: Recurrent tumor NOT visible, prostate bed irradiation only**	
Dose level	**Dose to prostate bed**	**Dose to tumor**	**Dose to prostate bed**	**Dose to tumor**
Level 1: 20 fractions	45.8 Gy in 2.29 Gy fractions	60.4 Gy in 3.02 Gy fractions	56.4 Gy in 2.82 Gy fractions	-
Level 2: 15 fractions	41.85 Gy in 2.79 Gy fractions	54.6 Gy in 3.64 Gy fractions	51.2 Gy in 3.41 Gy fractions	-
Level 3: 10 fractions	36.4 Gy in 3.64 Gy fractions	47.1 Gy in 4.71 Gy fractions	44.2 Gy in 4.42 Gy fractions	-

Serial PSA and serial correlative studies will be collected for two years post-treatment.

3.1.1 Dose Limiting Toxicity

The DLT period includes treatment period (4, 3 or 2 weeks depending on Dose Level) and the 3 following weeks. DLT is defined as any of the following:

- Grade 3 rectal, small bowel, or urinary toxicity that does not resolve to Grade 2 or less within 4 days with appropriate medical management.
- Other grade 3 in-field toxicities attributable to RT that do not resolve to Grade 2 or less within 4 days with medical management.
- Delays of more than one week in completing radiation treatment due to toxicity (cumulative duration of delay over the course of the entire treatment).

Additionally, toxicities that occur more than 3 weeks after completion of treatment and during the 24 month follow up period will be followed and scored but will not be used for escalation with the following exception. If Grade ≥4 late bowel or bladder injury is observed in 2 patients at any Dose level, dose escalation will be terminated and the dose below that dose level in which the toxicity was observed will be considered the MTD. Early and late toxicities used for assessment of DLTs should be attributable (possible, probable or definite) to radiation. Changes in sexual and erectile function will not be considered a DLT. MTD will be determined for each Arm separately.

3.1.2 Dose Escalation (Hypofractionation)

Dose hypofractionation escalation will follow the classical '3+3' trial design. If none of the first three patients in a Dose level experiences a dose-limiting toxicity during the DLT period, another three patients will be treated at the next higher Dose level. However, if one of the first three patients experiences a dose-limiting toxicity during the DLT period, three more patients will be treated at the same Dose level. Dose escalation continues until the final Dose level meets safety criteria or at least two patients among a Dose level group of three to six patients experience dose-limiting toxicities (i.e., ≥33% of patients with a dose-limiting toxicity at that Dose level).

Dose escalation (**Table 1**) will proceed in parallel in Arm 1 and Arm 2 in Dose level groups of 3–6 patients. The MTD is the dose level at which no more than 1 of up to 6 patients experience DLT during the DLT period, and the dose below that at which at least 2 (of ≤6) patients have DLT as a result of treatment.

Dose escalation will follow the rules outlined in the Table below for each Arm (1 or 2) independently as MTD and toxicities are expected to be different as a result of prior treatment.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter up to 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. UP to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the recommended phase 2 dose. 6 additional patients may be entered at the recommended phase 2 dose.

There will be no radiation dose modifications except planned Dose levels.

If a patient did not experience DLT and did not finish treatment, he will not be evaluable for toxicity and will be replaced in the dose level.

If patients develop side effects considered DLT, they will be taken off protocol treatment and may receive additional radiation as needed in standard fractionation to complete the treatment.

3.2 QUESTIONNAIRES (FOR ENGLISH SPEAKING PATIENTS ONLY)

(For English speaking patients only because questionnaires are not validated in other languages.)

Questionnaires will be collected on this trial, typically prior to visiting with the physician at each visit or within 1 week prior at the appropriate time points (**Appendix B**). Questionnaires may be completed in paper or electronically, but every effort will be made to have each patient complete questionnaires in the same format.

The timing of collection is noted on the study calendar in section **3.3**.

The total time anticipated to complete all questionnaires below is 20 minutes.

Once data are abstracted, questionnaires will be maintained in the research chart (print or electronic). If stored, electronically, these files will have restricted access and be accessible only to members of the study team. In the event that questionnaires are stored electronically, paper copies will be shredded.

3.3 STUDY CALENDAR

Procedure	Screening	Baseline[1]	Active RT treatment (2-4 weeks)	Follow Up, Months[7]		
				1	3	6, 9, 12, 15, 18, 21, 24
RT treatment			X			
Medical History/Records	X					
Confirmation of Pathology	X					
Physical exam, vital signs, ECOG	X	X		X		
CBC with differential and platelet count		X				
Biochemical profile [2]		X				
PSA	X	X			X	X[5]
Testosterone		X			X	X[5]
HIV	X					
CD4+ T-cell count for HIV positive patients	X					
Lymphocyte phenotype TBNK		X		X	X	X
Adverse Events		X	X	X	X	X
Concomitant Medications		X	X	X	X	X
mpMRI	X					X[8]
Whole body bone scan or 18F-NaF PET/CT imaging [9]	X					
CT of the abdomen and pelvis (if clinically indicated)	X					
Blood for correlative studies		X	X[3]	X	X	X[5]
Urine for correlative studies		X	X[3]	X	X	X[5]
Questionnaires [6]		X				X[4]
Archival tissue (if available, blocks or sections from prostatectomy or biopsies will be requested)		X				
Contact by phone call, e-mail or other NIH approved remote communication platform every 3 months once off treatment and unable to return for visits				X	X	X

1. Tests done at screening do not need to be repeated on baseline if performed in designated time frame prior to start of treatment
2. Biochemical profile: electrolytes, creatinine, BUN, alkaline phosphatase, ALT, AST, total and direct bilirubin
3. Sampling within 24 hours after the last dose of irradiation.
4. Every 6 months after treatment for 24 months
5. Every 3 months after radiation treatment for 24 months
6. Questionnaires: Expanded Prostate Cancer Index Composite (EPIC-26), SHIM, AUA-SI, PROMIS Depression SF4a, PROMIS Anxiety SF4a, PROMIS Psychological Impact Positive SF 4a, Decision Regret Scale. If the patient receives ADT, before initiation of ADT and within one week before starting radiation if ADT initiated more than one month prior to radiation. For English speaking patients only.
7. +/- 1 week for 1 month visit, +/- 2 weeks for 3 and 6 month visits. +/- 4 weeks for next visits. If subjects are not able or willing to come to NIH to FU visits, they will be followed every three months remotely (by phone call, e-mail for adverse events or other NIH approved remote communication platform), further tumor therapy and lab results. Lab results from outside laboratories are accepted. A clinical evaluation may be completed remotely with a member of the study team to determine if the physical exam, vital signs and ECOG may be omitted. A subject may be referred to a local providers for further assessment at the discretion of the investigator after a remote assessment (e.g., finding that requires clinical assessment/follow up). Subjects may also be referred to their local provider for labs. If biochemical failure per **6.3.1** is identified, the second measurement will be obtained for confirmation, ideally at the NIH Clinical Center. Imaging may be delayed, but will be completed as soon as feasible. Collection of research blood or urine will be omitted or collected in a delayed fashion.
8. Only at the 6 month follow up time point.
9. Scans may be omitted if eligibility can be determined from MRI or other imaging results including 18F-DCFPyL.

3.4 RADIATION THERAPY GUIDELINES

3.4.1 Treatment Planning

A CT simulation will be performed in the radiation oncology branch. The bladder should be comfortably full. Contrast is optional, but discouraged as it may distort anatomy and complicate heterogeneity corrections. If possible, additional imaging will be fused to the planning CT scan (i.e., MRI or other imaging on which tumor was identified).

Treatment planning definitions
GTV: gross tumor volume
CTV: clinical target volume
PTV: planning target volume

Arm 1: prostate bed and tumor boost treatment

- GTV lesion(s): image defined tumor
- PTV lesion(s): GTV lesion + 3 mm posterior and superior margin and 3-5 mm margin in all other dimensions (inferior, lateral, anterior) on the GTV lesion(s). An expansion of the

PTV to a maximum of 7mm in any direction due to imaging or treatment set up uncertainty may be used at the discretion of the treating radiation oncologist.

- CTV prostate bed: The CTV of the prostate bed will use consensus definitions [9, 41].
- Superiorly: The CTV should extend superiorly from the level of the caudal vas deferens remnant. In some cases, the vas deferens remnant may be difficult to visualize. In the absence of gross disease or seminal vesicle remnants, the superior limit of the CTV should extend at least 2 cm and need not extend more than 3-4 cm above the level of the pubic symphysis. The consensus definition calls for "inclusion of the seminal vesicle remnants, if present, in the CTV if there is pathologic evidence of their involvement. However, inclusion of any seminal vesicle remnants seen is recommended.
- Inferiorly: The CTV should extend inferiorly to > 8-12 mm inferior to vesicourethral anastomosis. With axial CT imaging, the anastomosis can often be seen in the retropubic region as one slice below the most inferior urine-containing image (the bladder must be modestly full). Inferiorly, the border of the CTV should be at least 8-12 mm below the VUA. If visualization of the VUA is problematic due to image quality or surgical clip artifacts, the inferior limit of the CTV can extend to a level just above the penile bulb.
- Anteriorly: Below the superior border of the pubic symphysis, the anterior border is at the posterior aspect of the pubis. The CTV extends posteriorly to the rectum where it may be concave at the level of the VUA. At this level the lateral border extends to the levator ani. Above the pubic symphysis the anterior border should encompass the posterior 1-2 cm of the bladder wall at the minimum and posteriorly it is bounded by the mesorectal fascia. At this level the lateral border is the sacrorectogenitopubic fascia or the obturator internus muscle.
- Posteriorly: The CTV extends posteriorly to the anterior rectal wall, but may be somewhat concave around the anterior-lateral aspect of the rectum to adequately encompass the prostate bed.
- PTV prostate bed: 7 mm margin on the prostate bed CTV. A 5-6 mm margin posteriorly may be used if necessary to meet dose constraints.

Arm 2. Prostate bed

- CTV prostate bed: As above
- PTV prostate bed: As above
- Dose to the PTV will be escalated as defined in Section **3.1.2**.

3.4.2 Dose constraints

Dose will be prescribed to 100%.
Max dose in PTV (highest dose PTV per plan): goal 107%, tolerated 115%
Min dose in PTV goal 95%
Contoured organs at risk: Bladder, bladder minus CTV, rectum, small bowel, femoral heads, penile bulb

DOSE LEVEL 1: 20 fractions

Normal organ limit **Dose level 1: 20 fractions**	No more than 50% volume receives dose that exceeds	No more than 70% volume receives dose that exceeds
Bladder minus CTV	52.6 Gy	33.8 Gy

Normal organ limit **Dose level 1: 20 fractions**	No more than 15% volume receives dose that exceeds	No more than 25% volume receives dose that exceeds	No more than 35% volume receives dose that exceeds	No more than 50% volume receives dose that exceeds
Rectum	60.0 Gy	56.2 Gy	52.6 Gy	48.2 Gy

Small bowel: V38 < 195cc (peritoneal cavity)
Femoral heads: No more than 10% to 45 Gy

DOSE LEVEL 2: 15 fractions

Normal organ limit **Dose level 2: 15 fractions**	No more than 50% volume receives dose that exceeds	No more than 70% volume receives dose that exceeds
Bladder minus CTV	47.8 Gy	31.1 Gy

Normal organ limit **Dose level 2: 15 fractions**	No more than 15% volume receives dose that exceeds	No more than 25% volume receives dose that exceeds	No more than 35% volume receives dose that exceeds	No more than 50% volume receives dose that exceeds
Rectum	54.4 Gy	51.1 Gy	47.8 Gy	44.5 Gy

Small bowel: V35 < 195cc (peritoneal cavity)
Femoral heads: No more than 10% to 42 Gy

DOSE LEVEL 3: 10 fractions

Normal organ limit **Dose level 3: 10 fractions**	No more than 50% volume receives dose that exceeds	No more than 70% volume receives dose that exceeds
Bladder minus CTV	41.4 Gy	27.5 Gy

Normal organ limit **Dose level 3: 10 fractions**	No more than 15% volume receives dose that exceeds	No more than 25% volume receives dose that exceeds	No more than 35% volume receives dose that exceeds	No more than 50% volume receives dose that exceeds
Rectum	46.8 Gy	44.1 Gy	41.4 Gy	38.7 Gy

Small bowel: V30 < 195cc (peritoneal cavity)
Femoral heads: No more than 10% to 36 Gy.

3.5 FOLLOW UP

After treatment completion, patients will be invited to Clinical center or referred to their local provider for follow up visit 1 month and 3 month post treatment, and subsequently every 3 months for 2 years. For evaluations during these visits, please, see Study Calendar **3.3**.

3.6 COST AND COMPENSATION

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by an insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

Participants will not be compensated on this study.

3.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.7.1 Criteria for removal from protocol therapy

- Completion of protocol therapy
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in section **3.1.1**
- PI discretion

3.7.2 Off-Study Criteria

- Completed study follow-up period
- Participant requests to be withdrawn from study
- Death
- Recurrence of prostate cancer
- PI discretion
- Non-compliance with follow-up visits or protocol mandated procedures
- PI decision to close the study
- Lost to follow up

3.7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

Supportive care with blood components, antibiotics, analgesics, and general medical therapy will be provided if the need relates to the performance of protocol assessments (imaging procedures) or to treat side effects of therapy. Every effort will be made to refer the patient to their collaborating physician in the community for care unrelated to the protocol therapy or procedures. There are no limitations on the type of non-investigational concurrent medications allowed, as in general, these patients do not receive additional therapy for prostate cancer after radiotherapy, with the exception of hormonal therapy, which may be delivered as per the current standard of care.

Radiation related side effects will be managed as per the current standard of care without restrictions in medications or procedures that may be performed for management.

4.1 ADT

We will allow patients to receive ADT on this trial after enrollment if deemed clinically appropriate, as the bulk of data suggests that it has no impact on the toxicities we have defined as DLTs in this trial.

ADT will be delivered on this protocol as per the current standard of care with the duration of therapy to be determined by the treating physician if indicated. This may include an anti-androgen, gonadotropin releasing hormone agonist, or the combination of both. As an alternative, patients may also receive ADT through their local care provider (medical oncologist, primary care physician, urologist) in collaboration with the research team.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Test/assay	Volume blood (approx)	Type of tube [a]	Collection point	Location of specimen analysis
ELISA/Bioplex analysis of predictive biomarkers	Plasma 8 mL	EDTA (lavender top)	See Study Calendar **3.3**	Dr. Citrin's Lab
Circulating DNA by whole genome sequencing with copy number analysis	5-10 mL	STREK	See Study Calendar **3.3**	Dr. Sowalsky's Lab
Exosome analysis (Surface receptor analysis by Nano FACS, RNA analysis)	Plasma 8 mL	EDTA (lavender top)	Study Calendar **3.3**	Dr. Jones Lab
Immune cell subsets and circulating endothelial cells by FACS	Whole blood 16 mL	CPT citrate	Study Calendar **3.3**	DTB Clinical Translational Unit
ELISA analysis of predictive biomarkers	Urine 15 mL	Sterile cup	Study Calendar **3.3**	Dr. Citrin's Lab
Whole genome sequencing	If available, tissue blocks or sections from prostatectomy or biopsies		Baseline	Dr. Sowalsky's Lab

a. Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

A number of exploratory correlative studies will be performed. These studies focus on two main areas: assessment of tumor radiation response (disease control) and prediction of normal tissue toxicity. Plasma and urine will be analyzed for the presence of markers of prostate cancer that

were initially described from proteomic analysis of prostate cancer patient's blister samples from protocol 09-C-0120. A number of candidate markers were identified during the discovery proteomic analysis, and the plasma samples from this trial will be used to test these markers without the complication of residual normal prostate tissue which may impact their suitability as a biomarker of cancer post-radiation. The post-prostatectomy setting provides an excellent minimal disease state to test candidate biomarkers.

5.1.1 Circulating immune cells during radiotherapy

During a course of radiotherapy, the target tissue is irradiated, including local immune cells. It is well known that radiation can cause changes in the local immune landscape, an effect that has been considered a major contributor to radiation injury [42-44]. Importantly, radiation can be considered immunosuppressive or immune activating depending on the field size, portion of the body targeted, and fractionation employed [45-48]. Recently, there has been tremendous interest in leveraging local and systemic immune changes caused by radiotherapy to enhance immunotherapy for cancer [49-54].

One previously observed change in immune cell subsets after irradiation is a change in the number and proportion of lymphocyte subsets, particularly CD4+ T cells and CD8+ T cells. Treatment induced lymphopenia can be quite striking in patients treated with combined radiation and chemotherapy [55, 56]. In elderly patients with high grade glioma, severe treatment related lymphopenia has been shown to correlate to inferior survival [57]. Treatment related lymphopenia has been observed in patients treated with post-prostatectomy radiotherapy with conventionally fractionated or moderately hypofractionated (2.35 Gy per fraction) approaches [58]. Although this report did not further classify lymphocyte subsets, we have accumulated data in anal cancer patients (**Figure 1**) and prostate cancer patients treated with radiotherapy on ROB protocols that suggests that CD4+ cells are disproportionally affected by radiotherapy +/- chemotherapy compared to other lymphocyte subsets. In our experience, the suppression of circulating lymphocyte subsets in prostate cancer patients is evident, but more variable in intensity and duration than what is seen when chemotherapy is delivered.

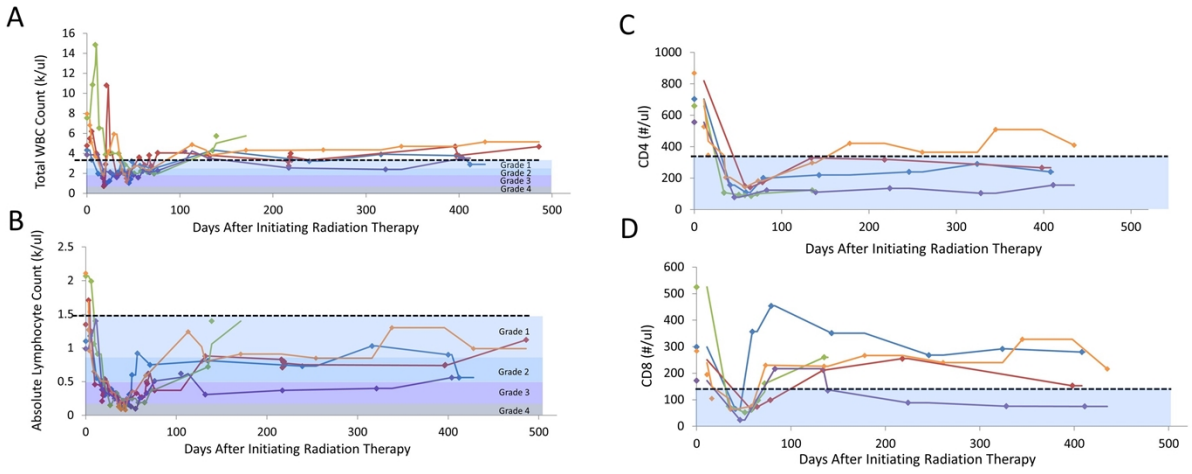

Figure 1. Treatment related lymphopenia. Immune cell subsets in HIV negative patients that receive Mitomycin-C, 5-fluorouracil, and radiation for anal cancer (protocol 11-C-0129). Radiation treatment was completed by 42 days. Total WBC (A), lymphocyte count (B), CD4+ T cells (C), and CD8+ T cells (D) were

assessed at multiple time points. Lines represent the trajectory for each patient. Toxicity grade (CTC AE) for each measure is noted.

Based on these findings, we are interested in more comprehensively evaluating lymphocyte subsets in this patient cohort. This trial will also allow us to begin to ask, in an exploratory fashion, if more aggressive hypofractionation and the volume irradiated alters the degree of lymphopenia. These are areas of substantial interest to the radiation oncology and immunotherapy community.

Macrophages also are known to play a critical role in radiation injury and the immune environment after irradiation [42-44, 59]. Radiation is known to deplete tissue resident macrophages, resulting in recruitment of bone marrow derived monocytes to repopulate [60]. Further, the polarization of monocytes/macrophages and lymphocytes is known to impact radiation toxicity [42, 61]. This trial will assess immune the subsets and polarization over time, in an exploratory fashion. We envision that these findings may spur additional clinical and laboratory research studies. Similarly, circulating endothelial cells will be analyzed before, at the end of, and one month after radiation in a purely exploratory fashion.

Immune cell subsets and polarization will be analyzed at several timepoints throughout the protocol in an exploratory fashion. Peripheral blood mononuclear cells (PBMC) will be assessed using multiparameter flow cytometry for conventional CD4+ T-cells, CD8+ T-cells, Tregs, monocyte subsets and MDSC populations. Assessment may include functional markers, i.e. PD-1, TIM-3, CTLA-4, CD40. PBMC will be analyzed for forward and side scatter, and a dump channel will be created to exclude cells expressing hematopoietic markers, such as CD45. Endothelial cells will be identified using co-expression of markers, such as CD31 and CD146 for mature endothelial cells and CD31 and CD133 for circulating endothelial progenitor cells (CEP). The cell populations will also be analyzed for viability using scatter profiles and a vital stain (e.g., Hoechst 33258). Percentages of stained cells will be determined and compared with appropriate negative controls.

5.1.2 Protein and RNA Analysis

In addition, protein analysis of plasma and urine will be conducted to explore if markers of senescence can predict for the risk of normal tissue injury after radiation. Dr. Citrin's laboratory has extensively studied normal tissue injury as a consequence of stem cell senescence or as a consequence of elaboration of molecules associated with senescence [42, 43, 62-66]. A number of these molecules known to be secreted after senescence, known as the senescence associated secretory phenotype (SASP)[67], will be evaluated with multiplex ELISA or similar quantitative protein assays in plasma and urine. Finally, plasma samples may be used to purify exosomes for further analysis via flow cytometry, protein assays, or RNA based approaches. These studies are all exploratory.

5.1.3 Samples

5.1.3.1 Plasma

Plasma will be sampled at baseline (within 2 weeks prior to radiation), at the end of radiation, 1 month after radiation, and every three months after radiation until 24 months.

Two 8 mL aliquots of blood will be obtained in EDTA containing tubes (Citrin/Jones) and one aliquot of whole blood in a STREK tube. CEC/CEP analysis will only occur at selected time points. For CEC/CEP and immune subset analysis, whole blood will be collected in two 8 mL

BD Vacutainer Cell Preparation Tubes (CPT/citrate) aliquots will be collected in citrate CPT tubes. CEC/CEP collections will occur prior to treatment (baseline, the last day of treatment, one month after treatment, and at one optional blood draw at a later follow up appointment. Following collection, EDTA tubes will be immediately placed on ice and transferred to the laboratory of Dr. Citrin for processing and storage, Building 10, B3-B100, Tel. 301-496-5457.

Samples for DTB Clinical Translational Unit (CPT tubes): Please notify the lab by email (Min-Jung Lee - leemin@mail.nih.gov; Akira Yuno – akira.yuno@nih.gov and Sunmin Lee – leesun@mail.nih.gov) that the blood draw has been scheduled. When the blood is being drawn, please call the Lab at 240-760-6330 to arrange for immediate pick- up.

Plasma will be stored and batched for analysis.

Plasma assays in Dr. Citrin's laboratory will include ELISA/Bioplex analysis of predictive biomarkers recently identified by the Citrin laboratory that correlate with tumor resistance to radiotherapy (70 analytes) or toxicity. Studies in Dr. Jones' laboratory will include surface receptor analysis by nanoFACS and RNAseq, for exosome and extracellular RNA analysis.

5.1.3.2 Urine

Urine will be sampled at baseline and every three months after SBRT.

At least 15 mL of urine will be obtained in a sterile collection cup. Following collection, urine will be transferred to the laboratory of Dr. Citrin for processing, measurement of creatinine concentration, and storage.

Urine will be stored and batched for analysis.

Urine assays will include ELISA analysis of predictive biomarkers recently identified by the Citrin laboratory that correlate with tumor resistance to radiotherapy (70 analytes identified in tissue, screening for detection will occur in urine and plasma).

5.1.4 MRI of erectile tissue

The erectile tissue lies just beneath the prostate. After prostatectomy, the penile bulb (base of the corpus cavernosa and spongiosa) is used as a landmark for the inferior portion of the radiation target volume. Patients treated with radiation to the prostate on 13-C-0119 have undergone pre-treatment and post-treatment MRI for evaluation of treatment response. On interesting component of this analysis has been the finding that erectile tissue shrinks substantially in patients treated with radiation and ADT. Because a treatment planning MRI is often obtained (as per standard) we have noted a major change in the erectile tissue from baseline until the time of radiation, corresponding to two months of treatment with ADT. Less change is found after the addition of radiation. Thus, much of the change observed in the erectile tissue may be due to ADT. We hope to expand these observations in an exploratory fashion to the post-prostatectomy setting. In this case, we plan to evaluate the width and imaging characteristics of erectile tissue at baseline and 6 months after treatment. The percent reduction in width of the erectile tissue and the volume of the penile bulb will be evaluated at 6 months after treatment. Changes in the imaging characteristics of erectile tissue in patients treated with ADT and without ADT will be evaluated separately.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management

system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All samples collected on this protocol and stored in Dr Citrin or Dr. Sowalsky's laboratories will be labelled and catalogued with LabMatrix. Labmatrix access within the laboratories is restricted to laboratory members processing and storing specimens.

Samples to be analysed in Dr. Citrin's and Dr. Jones' laboratory will be immediately processed and stored at -80º C until use in the ROB clinical specimen freezers.

Samples to be analysed by Dr. Sowalsky are processed appropriately and stored in secure freezers at -80° C until use.

Samples stored in the DTB Clinical Translational Unit laboratory will be immediately processed. Specimens are stored in secure freezers at optimal temperatures for the sample type, i.e. -80° for plasma and liquid nitrogen for viably frozen cells. All freezers are continually monitored. Samples are 2D barcoded without identifying information. Access to the patient database and code key is limited to defined laboratory personnel, and access is only possible on limited computers with a password-restricted login.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

Blood and urine samples may be subjected to protein or RNA analysis in the future to describe novel prognostic or predictive biomarkers.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

5.3 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.3.1 Description of the scope of genetic/genomic analysis

5.3.1.1 Tumor whole genome sequencing

If available, tissue blocks or sections from prostatectomy or biopsies will be requested to perform whole genome sequencing or RNA analyses. This data will be used for comparison to circulating DNA analyses as outlined below or as a compliment to exosome studies. These will be exploratory analyse to compare the circulating DNA copy number results or exosome analyses to previously resected tumour tissue. These analyses may only be carried out if circulating DNA analyses or exosome analyses are promising as defined below.

5.3.1.2 Circulating DNA

The anticipated analysis on circulating DNA will include whole genome sequencing with copy number analysis, performed by Dr. Sowalsky's laboratory.

We will use the approach of whole genome sequencing with copy number analysis of circulating cell free DNA. Copy numbers that vary from expected at baseline will be used as a tumor marker in longitudinal sample assessment (copy number abnormality at baseline will be compared to a reference region at follow up time points). This is purely an exploratory analysis to determine feasibility of the approach.

5.3.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

- Samples will be bar coded for storage and personal identifiers will be removed. Names and demographic data will be linked to bar codes in the password protected LabMatrix database. This data will be accessible only by the PI and the research nurses within the ROB.
- Samples provided to CCR collaborators will be barcoded. No personally identifiable information will be released to Dr. Sowalsky.
- Data from genomic and genetic data will be deposited in dbGAP and released in accordance with NIH guidelines.
- As part of study efforts to provide confidentiality of subject information, this study will obtain a Certificate of Confidentiality. Please refer to Section **10.4**.

5.3.3 Management of Results

We plan to contact participants if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of secondary findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Unless requested otherwise, participants will be contacted at this time with a request to provide a sample to be sent to a CLIA certified laboratory.

5.3.4 Genetic counseling

If the research findings are verified in the CLIA certified lab, the participant will be offered the opportunity to schedule a visit, in person (at our expense) or via telemedicine on an NIH approved platform to have genetic education and counseling with the NCI Genetics Branch to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at participant's expense).

This is the only time during the course of the study that secondary findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Participants will be requested to maintain up to date contact information to enable future contact.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted

data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Document AEs from the first study intervention through at least 30 days after the last study treatment administration. Beyond 30 days after the last study treatment administration, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study (except for a rising serum PSA)
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.1 Data Elements/Information

The presence of any pre-administration event (baseline signs and symptoms present just before radiation) will be recorded in the clinical research database:

- The date and time of evaluation
- The onset time
- The resolution time or duration
- Action taken
- Status of symptom
- Intensity
- Date of birth
- Demographic data
- Prior and concurrent medications
- Medical history and concurrent diseases
- Results of baseline tests: physical examination; vital signs; lab tests
- Questionnaire scores.

Personal data (including contact information) will be collected with the subject's permission and only to the extent that is necessary for the purposes of the study.

Exceptions to data collection:

- As androgen deprivation therapy (ADT) is given as part of standard of care, adverse events/toxicities that occur related to ADT will not be collected or reported.

The following data will be collected and evaluated according to the Study Calendar (Section **3.3**):

- Physical examination
- Concomitant medications
- AEs
- Use of ADT
- Presence or absence of biochemical relapse
- Questionnaire scores

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded and linked data in an NIH-funded or approved public repository.
- Coded and linked data in BTRIS (automatic for activities in the Clinical Center)

How and where will the data be shared?

- An NIH-funded or approved public repository: clinicaltrials.gov, dbGAP
- BTRIS (automatic for activities in the Clinical Center).
- Publication and/or public presentations.

When will the data be shared?

- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 3 months after treatment with a serum PSA measurement as per the study calendar. Response will be assessed based on PSA.

6.3.1 Response Criteria

Biochemical control: Biochemical control will be defined as a post-radiation PSA that does not rise beyond 0.1 ng/dL.

Biochemical progression/biochemical failure: Serum PSA more than 0.1 ng/dL. At least 2 PSA measurements separated by 1 month are needed to confirm biochemical failure. If a PSA pre-

treatment was >0.1 ng/dL, a post-treatment value >0.1 ng/dL will not be considered biochemical progression if subsequent values continue to trend downwards after completion of radiation.

6.3.2 Biochemical Progression-Free Survival

Biochemical progression free survival (bPFS) will be evaluated on this trial as a secondary endpoint. bPFS is defined as the duration of time from start of treatment to time of PSA progression or death, whichever occurs first. PSA progression (also known as biochemical failure) is defined based on elevation of PSA beyond 0.1 ng/dL. A total of 2 rising PSA values separated by at least 1 month are required to document biochemical recurrence. If the PSA remains elevated, the date of failure will be backdated to the first PSA measurement that exceeded 0.1 ng/dL. Only PSA values collected at the NIH Clinical Center will be used to evaluate disease status given the variability in ranges obtained in different laboratories.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. 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. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis, at a minimum bi-weekly, when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical Hypothesis

8.1.1 Primary Objective

- Define the maximum tolerated dose (MTD) of image guided, hypofractionated, focally dose escalated post-prostatectomy radiation. Assessment of toxicities for dose escalation will occur through 3 weeks after radiation.

8.1.2 Secondary Objectives

- Define the toxicity profile of image guided, hypofractionated, focally dose escalated post-prostatectomy radiation. Assessment of acute toxicities for dose escalation will occur through 3 weeks after radiation.
- Describe the rate of PSA control (biochemical progression free survival, bPFS) at 1 and 2 years after treatment with image guided, hypofractionated, focally dose escalated post-prostatectomy radiation. We hypothesize that the rate of PSA control (bPFS) will compare favorably to the rate obtained with salvage radiation previously published series.
- Describe the effects of image-guided, focally dose escalated prostate bed radiation on patient reported outcomes (SHIM, AUA Symptom Index, EPIC-26). We hypothesize a reduction in quality of life (urinary and gastrointestinal) after radiation that persists for several months but returns to baseline in most patients by 2 years after treatment. We hypothesize that erectile dysfunction will progress without improvement over time.

8.2 Sample Size Determination

The planned sample size is a minimum of 4 patients (2 per arm) and a maximum of 48 (24 patient's maximum per arm) based on the 3+3 design detailed in **3.1.2**. An expansion of six additional patients can occur at the MTD in each arm as noted in **3.1.2.** Estimates for sample size

are based on the assumption that all dose levels will accrue a maximum of patients, thus sample size is likely over-estimated. We anticipate accrual will require 30 months.

8.3 POPULATIONS FOR ANALYSES

All enrolled patients that initiate treatment with radiation will be considered evaluable for toxicity, unless they are removed from protocol treatment for a reason other than a DLT (such as illness unrelated to radiation). Patients that complete at least one post-treatment PSA will be considered evaluable for biochemical control and biochemical relapse free survival. Patients that complete QoL measures at 3 months will be considered evaluable for these measures.

8.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with radiation, unless they are removed from protocol treatment before the end of the assessment period for a reason other than a DLT (such as illness unrelated to radiation).

Evaluable for response: Only those patients who complete radiation and have PSA measured after radiation will be considered evaluable for response. These patients will have their response classified according to the definitions stated earlier.

8.4 STATISTICAL ANALYSES

8.4.1 General Approach

This is a phase I study. There are two arms to this study for 1) patients with prior prostatectomy, rising PSA, and visible tumor on imaging, and 2) patients with prior prostatectomy with no visible tumor on imaging (rising PSA or indications for adjuvant treatment). Arms are enrolled and escalated separately. Dose level groups of 3-6 patients are evaluated at each dose level. The dose level on which 2 patients experience unacceptable toxicity is considered to have exceeded the MTD. The next lower dose level on which no more than 1/6 patients experience unacceptable toxicity is considered the MTD for each arm of the study.

8.4.2 Analysis of the Primary Endpoints

The primary endpoints will be to define the MTD of this approach.

8.4.3 Analysis of the Secondary Endpoint(s)

- Define the toxicity profile of image-guided, focally dose escalated prostate bed radiation in patients with a local or regional recurrence of prostate cancer after prostatectomy. All AEs except grade 1 with attribution to protocol treatment will be collected in tabulated way and analyzed.
- The rate of PSA control (biochemical progression free survival, bPFS) will be estimated by the Kaplan-Meier survival analysis and effects of clinical variables on bPFS will be assessed by the Cox proportional hazards model.
- The quality of life scores will be summarized at baseline and for each visit. Linear mixed effects model will be used to model quality of life scores at baseline and during and after treatment in which random intercept and random slope are used to account for patient-specific trajectory of quality of life scores. Changes of quality of life scores during and after treatment will be calculated from the estimated linear mixed effect model. The

similar analytical approach will be performed for depression scores and correlated with quality of life scores.

8.4.4 Safety Analyses

Adverse events will be coded as per the CTCAE v4.0. Each AE will be coded once for each patient based on the maximum severity during the assessment period. The relationship of AEs to study intervention will be recorded in addition to the onset date, end date, severity, expectedness, and outcome.

Dose-limiting toxicities are defined as follows (during treatment and within the first three weeks after treatment):

- Grade 3 rectal, small bowel, or urinary toxicity that does not resolve to Grade 2 or less within 4 days with medical management
- Other grade 3 in-field toxicities attributable to radiation that do not resolve to Grade 2 or less within 4 days with medical management
- Delays of more than one week in completing radiation treatment due to toxicity (cumulative duration of delay over the course of the entire treatment).

Additionally, toxicities attributable to radiation after the DLT assessment period (3 weeks after radiation is completed) will be followed and scored but will not be used for escalation with the following exception. If Grade $\geq$4 late bowel or bladder injury (late toxicity is defined as occurring >90 days after completion of radiotherapy) is observed in 2 patients in any Dose level group, dose escalation will be terminated and the dose below that dose level in which the toxicity was observed will be considered the MTD. Early and late toxicities used for assessment of DLTs should be attributable (possible, probable or definite) to radiation. Sexual and erectile dysfunction as a result of radiation will not be considered a DLT. Note that MTD will be determined for each Group separately.

Dose-limiting toxicities are defined as per section **3.1.1**.

8.4.5 Exploratory Analyses

- Evaluate decision regret at the time of enrollment and at the end of follow up relating to the primary treatment for prostate cancer to determine if successful salvage mitigates decision regret. The percentage of patients scored as having decision regret will be compared to the percentage of patients not exhibiting decision regret (regarding the initial surgery). It is hypothesized the effective salvage therapy will mitigate decision regret expressed at the time of enrollment.
- Explore candidate biomarkers of radiation toxicity in blood and urine of patients receiving image-guided, hypofractionated, focally dose escalated post-prostatectomy radiation. Protein and metabolic biomarkers will be assessed in collected specimens.
- Explore MRI imaging parameters of erectile tissues in regards to testosterone and erectile dysfunction. MRI imaging of erectile tissue will be compared to evaluate for changes in volume and tissue characteristics after radiotherapy.
- To compare dosimetry of standard compared to biological optimization algorithms for treatment planning of post-prostatectomy radiation. Treatment planning will be optimized

with both approaches to allow comparison of normal tissue complication probability curves. Results will be reported in a descriptive fashion.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

9.2 INCLUSION OF WOMEN AND MINORS

Women are excluded from this trial as prostate cancer does not occur in females. Boys younger than 18 are excluded because they do not develop prostate cancer with the exception of rare histologies, such as rhabdomyosarcoma, for which radiation alone and the radiation doses chosen here are not an appropriate treatment.

9.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

9.3.1 Benefits

The potential benefits of trial participation relate to potential curative treatment of recurrent prostate cancer. As information is gathered from this trial, clinical results will be shared with patients as they become available. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found during the course of the research, which may affect a patient's willingness to participate further, will be explained.

Confidentiality of information concerning participants will be maintained, including in all publications and presentations resulting from this study. Names of participants and/or material identifying participants will not be released without permission, except as such release is required by law. Records at the National Cancer Institute are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

9.3.2 Risks

9.3.2.1 Radiation Therapy

The risks of participation are primarily those of toxicity from irradiation. Subjects in cohort 1 will be exposed to up to 60.4 Gy of radiation in 20 treatment sessions, or up to 54.6 Gy in 15 sessions or up to 47.1 Gy in 10 sessions. Subjects in cohort 2 will be exposed to up to 56.4 Gy in 20 treatment sessions, or 51.2 Gy in 15 sessions, or 44.2 Gy in 10 sessions.

The available studies support that hypofractionated radiation can be safe and effective in this setting. Based on the experience of standard post-prostatectomy radiation, there is a moderate risk of low grade, chronic bowel or bladder toxicity and a decline in erectile function. There is a small risk of more severe injury to the bowel or bladder that could require a procedure (cystoscopy, endoscopy) for evaluation or treatment. Although extremely rare, patients receiving

post-prostatectomy radiation may have severe toxicity that could cause urinary obstruction, worsen post-surgical urinary incontinence, or require surgical correction. It is anticipated that the risks of this schedule of irradiation will be similar although they may be more or less in frequency or intensity. In addition, there smaller risks from blood draw, such as the risk of pain, infection or bleeding.

9.3.2.2 Research Blood Collection Risks

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop. Up to 62 mL of blood may be collected at baseline and up to 52 mL during each follow up visits.

9.3.2.3 MRI risks

The risks associated with MRI are discomfort and claustrophobia.

9.3.2.4 Gadolinium Risks

The most common side effects include injection site pain, nausea, itching, rash, headaches and dizziness. Serious but rare side effects such as gadolinium toxicity and nephrogenic systemic fibrosis, or NSF, are most often seen in patients with severe kidney problems.

9.3.2.5 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

9.3.2.6 Non-Physical Risks of Genetic Research

Risk of receiving unwanted information.

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Patients will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

Risk related to possibility that information may be released.

This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document.

9.4 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

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

10.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

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

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

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

10.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. 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 design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a

secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

11 COMMERCIAL DEVICE INFORMATION

There will be no IDE obtained for the use of any of the commercial agents used in this study.

This study meets the criteria under category #1 for exemption for an IDE as this investigation involves the use of an already cleared medical device in which the device is used or investigated in accordance with the indications in the cleared labeling.

11.1 SOURCE

The radiation device is a commercial device that will be located in the NIH Clinical Center.

11.2 TOXICITY

Refer to section **9.3.2.1**.

11.3 ADMINISTRATION PROCEDURES

Refer to section **3.1**.

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13 APPENDICES

13.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

13.2 APPENDIX B: QOL QUESTIONNAIRES

QoL questionnaires will be maintained in a separate document/manual.