FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

Radiation Enhancement of Local and Systemic Anti-Prostate Cancer Immune Responses

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Study Summary

Title	*Radiation Enhancement of Local and Systemic Anti-Prostate Cancer Immune Responses*
Short Title	*Neutron radiation immune response in CSPC*
Protocol Number	*CC9938*
Phase	*Pilot*
Methodology	*2 Arm Randomized*
Study Duration	*2 yrs*
Study Center(s)	*University of Washington Medical Center*
Objectives	*The primary objective of this study is to assess the systemic immune effects of radiation with high relative biological effectiveness (RBE) in the form of neutron radiation, in combination with androgen deprivation therapy (ADT) in patients with newly diagnosed castration sensitive prostate cancer (CSPC). Patients on ADT will be randomized to either standard of care abiraterone + prednisone, or same plus radiation to 1-3 sites of metastatic disease and undergo blood draws at multiple time points. The primary endpoint will measure percent change in peripheral blood effector T-cells (CCR7-/CD45RO-)-post-therapy (3 months after start of ADT, which is also 1 month post-radiation in the radiation arm).*
Number of Subjects	*30 evaluable subjects*
Diagnosis and Main Inclusion Criteria	*Patients diagnosed with CSPC, beginning ADT and abiraterone + prednisone as first line therapy, eligible for neutron radiation therapy to at least one metastatic site of disease. Site of radiation does not have to be symptomatic.*
Study Product, Dose, Route, Regimen	*All treatments are approved standard of care for patients with CSPC, including ADT, abiraterone, prednisone, and neutron radiation.*
Duration of administration	*ADT and abiraterone will be given per standard clinical care for at least 6 months or until patients develop progression (unless treating oncologist feels patient is still deriving benefit from therapy), or intolerable toxicity. Neutron radiation treatments will be given standard of care as well, for a maximum of 5 daily radiation treatments.*
Statistical Methodology	*In order to detect an estimated 10% increase in peripheral blood effector T-cells (CCR7-/CD45RO-)in the neutron arm versus the no radiation arm with a two-sided type I error rate of 5% and 80% power, while assuming a 10% standard deviation in each patient group, around 15 patients are required per arm.*

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1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

Prostate cancer has seen limited success with immunotherapy, although there is suggestion of clinical activity:

Prostate cancer remains the most common cancer in men in the United States and the number three cause of cancer death[1]. Most patients initially present with disease that is sensitive to androgen deprivation therapy (ADT), and for patients who present with metastatic disease, LHRH agonists such as leuprolide or LHRH antagonists such as degarelix are commonly used as first line treatment. Recent trials LATITIDE and STAMPEDE showed that the addition of abiraterone plus prednisone to ADT in castration sensitive prostate cancer (CSPC) as part of first line therapy can improve survival over ADT alone.[2,3] Response rate to first line ADT is high but many patients eventually develop metastatic castration resistant prostate cancer (mCRPC) and therapeutic options become more limited. Although immense strides have been made in systemic therapy options for mCRPC, there has been limited success with immunotherapy[4]. Sipuleucel-T remains the only FDA approved immunotherapy for mCRPC[5], with two phase III trials comparing ipilimumab versus placebo unable to improve overall survival[6,7]. There was, however, evidence of clinical activity, with superior progression free survival in the ipilimumab arm in both trials. The trial by Kwon *et al.*, gave standard photon radiation to a bony site prior to ipilimumab, based on the hypothesis that radiation could stimulate an immune response and enhance the efficacy of ipilimumab[6,8]. Multiple trials are ongoing in mCRPC testing new immunotherapy agents, combinations of immunotherapy agents, as well as immunotherapy plus radiation[4].

Radiation therapy could potentially improve survival in metastatic prostate cancer:

Historically, the only role of radiation therapy in metastatic prostate cancer has been for palliation, either to address existing symptoms (such as pain from a metastasis in the bone), or prevent future symptoms (such as radiation to the spine to prevent spinal cord compression). However, two trials were published in 2018-2019 that suggest radiation could improve survival in oligometastatic prostate cancer (limited number of metastatic sites). The STAMPEDE trial randomized men with newly diagnosed metastatic prostate cancer to standard of care systemic therapy (ADT with or without chemotherapy) plus or minus radiation to the prostate[9]. For patients with limited metastatic disease burden, radiation to the prostate improved overall survival (HR 0·68, 95% CI 0·52–0·90; p=0·007; 3-year survival 73% with control vs 81% with radiotherapy). Another trial called SABR-COMET randomized patients with oligometastatic cancers (1-5 metastatic lesions) and controlled primary tumors to standard of care systemic therapy, versus systemic therapy plus aggressive radiation (stereotactic ablative radiation therapy), and found radiation improved median progression-free survival to 12.0 months from 6.0 months (HR = 0.47, P = .0012), and median overall survival to 41 months (95% CI = 26 months–not reached) from 28 months (95% CI = 19–33 months, HR = 0.57, P = .090)[10]. Prostate cancer was one of cancer types included in the SABR-COMET trial. Multiple additional randomized trials are ongoing internationally to investigate the role of radiation in prolonging survival in metastatic prostate cancer.

ADT has been shown to have an effect on the immune response in prostate cancer:

Although the primary anti-tumor effect of ADT on prostate cancer is directly inducing cancer cell apoptosis, ADT also has an effect on the adaptive immune response.[11] Patients on ADT show an increase in naïve CD4+ T-cells in peripheral blood of almost 50% by 1 month after starting ADT, likely

attributable to increased thymic output.[12] At the same time, CD4+ and CD8+ T-cells show increased proliferation in response to stimulation, suggesting the T-cells are not only increasing in number but also responsiveness. This increased proliferation of around 10% was statistically significant in the effector cell population at 1 month (Figure 1). However, after 2 years of ADT, a decrease was seen in Th1 and Th17 effector memory subsets in peripheral blood. Therefore, ADT may induce an anti-tumor immune response early in the treatment course but eventually contribute to an immunosuppressive environment.[13]

Standard photon radiation combined with immunotherapy augments anti-tumor activity in solid tumors:

Radiation causes release of tumor antigens and cytokines into the tumor microenvironment, which leads to an inflammatory response and infiltration of immune cells including T-cells (both cytotoxic and regulatory), dendritic cells, macrophages, and myeloid-derived suppressor cells (MDSCs)[14]. Peripheral blood from patients with metastatic melanoma receiving radiation to a single site of disease while on ipilimumab show that radiation induces an increase in percentage of CD4+ T-cells, ratio of CD8+ T-cells to Tregs, decrease in MDSCs, and increase in HLA-DR expression on monocytes[8,15]. Clinical reports have been published with radiation to one tumor causing an abscopal effect that leads to systemic regression of disease outside the radiation field, even in patients who have previously progressed on immunotherapy[8,16,17]. Multiple ongoing trials are testing the combination of immune check-point blockade with radiation therapy in a variety of disease sites (NCT01952769, NCT02289209, NCT02298946).

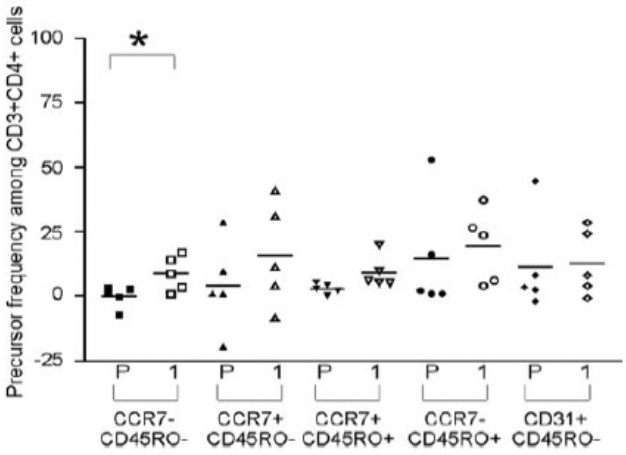

Figure 1. Change in peripheral blood in 12 patients on ADT showing statistically significant increase in effector T-cells (CD4+/CCR7-/CD45RO-) after 1 month compared with pretreatment (P). Cells were stimulated with anti-CD3 and anti-CD28 antibodies. Adapted from Morse and McNeel. Human Immunology 71 (2010): 496-504.

Strengths and limitations of standard photon radiation as an immune-adjuvant:

All of the published data on radiation and its ability to augment response to immunotherapy have been with standard photon radiation, which is sparsely ionizing with a relative biological effectiveness (RBE) of ~ 1, compared to 1.1 for protons and 3 or more for densely ionizing radiations such carbon ions and fast neutrons.[18-20] While the mainstay of radiation treatment worldwide is still photon therapy, particle therapy is increasingly prevalent. The main attraction of particle therapy is the ability to improve dose conformality by depositing most of the radiation in the tumor and less radiation in normal tissues[21]. Although protons are more widely available than carbon or other high RBE particles, protons have similar biologic efficacy as standard photon therapy (proton RBE ~ 1.1), but fast neutrons and carbon ions have higher RBE of 3 or more.[19-21] Higher RBE particles have a unique ability to overcome hypoxia, cell cycle radiosensitivity, and other effects that enable some tumors to resist standard photon treatments. For example, *p53* mutated cells, which are associated with more aggressive prostate cancers[22], are relatively radioresistant to standard photon therapy but more susceptible to high RBE radiation, meaning it is likely that high RBE radiation has a greater impact on the tumor microenvironment and immune milieu[23].

Immunologic effects of high-RBE radiation compared with standard photon radiation:

High RBE particle therapy such as neutrons cause qualitative changes in the sub-cellular distribution and types of DNA damage, and have the potential to shift cells from an apoptotic cell death mode towards the more inflammatory, and possibly immunogenic, mitotic catastrophe and necrotic cell death modes.[24-26] High RBE therapy may cause a more immunogenic tumor microenvironment via release of more tumor neoantigens[27,28]. Mouse total body radiation experiments in the 1970s with neutrons versus standard photons found that the degree of immunosuppression was dependent on the LET of the radiation (neutrons have high LET), and mortality from total body radiation differs based on LET, for the same delivered radiation dose[29].

Immunologic effects of standard photon therapy on patients receiving radiation for prostate cancer:

There is limited data on the immune response of patients with prostate cancer receiving palliative radiation with standard photon therapy alone, without immunotherapy. Schaue *et al*., looked at peripheral blood lymphocytes from 20 patients with localized prostate cancer receiving standard photon radiation treatment. CD8+ T-cells able to bind survivin, which is overexpressed in prostate cancer, was detected in peripheral blood and increased after therapy in some patients[30]. Nesslinger *et al*., also looked at peripheral blood in 73 patients with localized prostate cancer undergoing standard treatments including standard photon radiation[31], and found that 13.8% of patients developed treatment associated autoantibodies detected via Western blot probing against LNCaP cell lysates. It is possible to detect changes in immune response in peripheral blood after localized radiation therapy for prostate cancer.

Rationale for combination of ADT, abiraterone+prednisone, and neutron radiation for enhancing the systemic immune response to prostate cancer:

Both ADT and radiation have been shown to modulate the systemic anti-tumor response. Although ADT might contribute to an anti-tumor immunologic reaction earlier in the treatment course, the effects may become more immunosuppressive over time.[11] Radiation can also be immunosuppressive given that lymphocytes are extremely sensitive to radiation killing, but the right dose of radiation to a limited area at the right time can act similarly to a "vaccine" to enhance or induce an immune reaction.[14] Adding a short course of high-intensity radiation early in the ADT treatment course may enhance and prolong the anti-tumor immunologic changes seen with ADT.

Although ADT with LHRH agonists or antagonists alone have been standard of care first-line therapy in CSPC for years, results from LATITUDE and STAMPEDE show that the addition of abiraterone + prednisone improves survival over ADT alone even in the first-line setting, and this treatment combination is increasingly used to treat patients with newly diagnosed metastatic prostate cancer.[2,3] Little is currently known regarding the immunologic effects of abiraterone + prednisone and there are concerns about immunosuppressive effects of the low dose prednisone given with abiraterone. However, a randomized phase 2 trial of sipuleucel-T with abiraterone + prednisone showed that concurrent administration of both therapies did not blunt or alter the immune parameters seen with sipuleucel-T.[32] In vitro studies of abiraterone show it sensitizes prostate tumor cells to T-cell mediated lysis via an androgen-receptor dependent pathway,[33] and can act synergistically with immunotherapy in a mouse model to control prostate tumors.[34] Large series of patients treated with checkpoint inhibitor immunotherapy needing immunosuppressive therapy (prednisone taper) to manage toxicity have shown that treatment efficacy is not diminished in patients who received immunosuppressive therapy versus those who did not, suggesting low dose prednisone does not negate the changes and benefits of immunotherapy.[35,36] It is also plausible that the addition of abiraterone to the treatment regimen induces a more robust immune response, as testosterone levels are lowered approximately 1-2 logs lower than can be achieved with LHRH therapy alone[37]. Therefore, we propose this study to look at the ability of neutron

radiation to enhance the anti-tumor immunologic changes seen with ADT plus abiraterone and prednisone in first line therapy for advanced castration sensitive prostate cancer.

Rationale for peripheral blood effector T-cells as surrogate endpoint for systemic immune response:

Various studies have reported on correlations between changes in peripheral blood immune cell subsets and clinical outcomes, with no current consensus on the most important population changes for prognosis prediction. However, multiple studies have shown that an increase in peripheral effector T-cells is correlated with improved clinical outcomes. Sander *et al*. looked at peripheral blood of 84 patients with acute myeloid leukemia who were in first remission after chemotherapy, starting maintenance immunotherapy with low dose IL-2 and histamine dihydrochloride[38]. When comparing peripheral cell counts before and after the first treatment cycle (C1D1 versus C1D21) as shown in Figure 2, patients who did not ultimately develop disease relapse had an increase in effector T-cells (CCR7-/CD8 T-cells) of approximately 10%, versus no significant change in peripheral effector T-cell percentage in patients who did develop disease relapse.

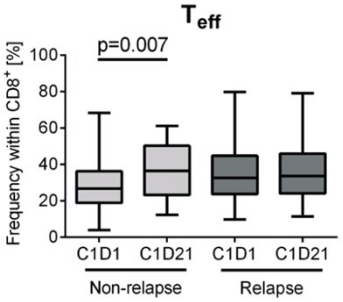

Figure 2. Change in percent CCR7-CD8 effector T-cells after first cycle of immunotherapy. Adapted from Sander FE et al. Oncotarget. 2016.

Immune profiling of peripheral blood from patients on checkpoint inhibitor immunotherapy has not been widely explored. Huang *et al.* looked at peripheral blood of 29 patients with metastatic melanoma receiving anti-PD-1 antibody pembrolizumab[39]. At 3-weeks into therapy, frequency of Ki67+ CD8 effector T-cells peaked and then declined, with highest percent of Ki67+ CD8 T-cells around weeks 3-6 after start of pembrolizumab (Ki67+ CD8 T-cells increased from around 7% at baseline to around 14-19% at weeks 3-6 post-pembrolizumab). The increase in Ki67 expression was mostly seen in the PD-1+ versus PD-1 negative CD8 effector T-cells, with Ki67 increasing from around 9% at baseline to around 23% at 3-weeks (peak response) in the PD-1+ CD8 T-cells population, and non-statistically significant increase in Ki67+ cells in the PD-1 negative CD8 T-cell population (Figure 3). Clinical response was correlated with the fold change of PD-1+ Ki67+ CD8 effector T-cells after anti-PD-1 therapy, adjusted for baseline tumor burden. Patients with longer PFS typically had a low tumor burden and higher fold change in Ki67+ CD8 effector T-cells. Using an arbitrary cutoff of 2.2 fold change in Ki67+ PD-1+ CD8 T-cells after anti-PD-1 therapy at weeks 3-12 compared to baseline, response rate was 40% (8/20) for patients with Ki67 change above the fold, and 29% (2/7) for patients below the fold. T-cell receptor repertoire was compared between pretreatment tumor infiltrating T-cells and peripheral blood CD8 T-cells. Across 6 patients, at peak Ki67 expression after

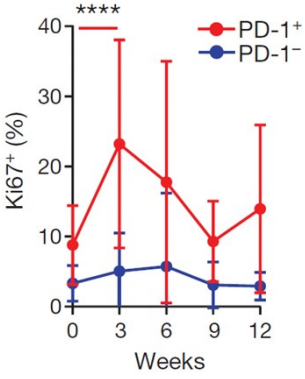

Figure 3. Change in percent Ki67+ CD8 T-cells after start of pembrolizumab therapy (n=29). Red line=PD-1+ cells, blue line=PD-1 negative cells. Adapted from Huang AC et al. Nature 2017 (545):60-65.

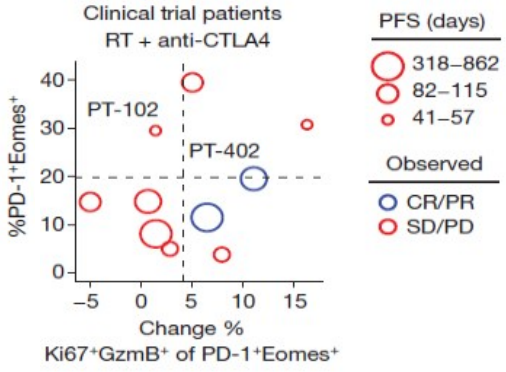

Figure 4. Change in peripheral blood in 10 patients with metastatic melanoma receiving photon radiation to a single index lesion and 4 cycles of anti-CTLA4 therapy. Percentage of Eomes+PD-1+ CD8 T cells in post-treatment blood vs change in%PD-1+Eomes+CD8 T cells that are Ki67+GzmB+ after treatment. Each circle represents a patient. Progression-free survival (PFS) is proportional to circle size and quadrant boundaries are average values for patients under the mean PFS. Concordance index of the random forest model is 0.59. Adapted from Twyman-Saint Victor C et al. Nature 2015 (520):373-377.

anti-PD-1 treatment, 14 clones were present among the top 10 clones in both tumor and blood, supporting the notion that Ki67+ T-cells in the blood are reinvigorated by anti-PD-1 therapy and contain T-cell clones that are also present in the tumor. This study shows that it is possible to detect immunologic changes in peripheral blood after treatment with anti-PD-1 therapy, and changes in Ki67+ CD8 effector T-cells may peak around weeks 3-6 and be related to clinical outcome.

Twyman-Saint Victor *et al*. reported on 22 patients with metastatic melanoma treated with hypofractionated photon radiation to a single index lesion, followed by 4 cycles of anti-CTLA4 antibody ipilimumab[15]. Radiation was found to increase the diversity of the T-cell receptor repertoire while anti-CTLA4 therapy inhibited T-regulatory (Treg) cells and increased the CD8/Treg ratio. For 10 patients with available pre- and post-treatment blood, two had partial responses in unirradiated tumors and progression-free survival significantly longer than the median. For both of these patients, the percentages of Ki67+ GzmB+ cells increased in PD-1+ Eomes+ CD8 effector T-cells after treatment (6-12% increase), while the proportion of PD-1+ Eomes+ T-cells remained at or below the mean (Fig. 4). In contrast, patients with a high percentage of PD-1+ Eomes+ T-cells post-treatment did not have partial responses and had a short progression-free survival, regardless of reinvigoration. Peripheral blood was collected post-radiation treatment and pre-anti-CTLA4 therapy, as well as every 3 weeks with anti-CTLA4 therapy for 4 cycles.

Taken together, the studies above suggest that it is possible to detect changes in effector T-cell populations in the peripheral blood in response to therapies that affect the immune system, and increase in effector T-cells may be correlated with improved clinical outcomes.

2 Study Objectives

Primary Objective:

Compare the percent change in peripheral blood effector T-cells (CCR7-/CD45RO-) between the ADT+ abiraterone arm and the ADT+ abiraterone+ radiation arm, measured at immediately prior to starting abiraterone, and 1 month after start of abiraterone (which is also about 1 month after radiation treatment in the radiation arm).

Secondary Objectives:

- To evaluate the rate of patients with undetectable PSA (<0.2 ng/mL) at 6-months after start of abiraterone.
- To evaluate the frequency and severity of toxicities as measured by CTCAE 4.0.

Exploratory Objectives:

- Changes in peripheral blood immune cell subpopulations measured via multi-parameter flow cytometry at all specimen collection time points:
    - Important T-cell subsets using markers such as: CD3/CD8/CD4/Foxp3/CD45RA/CD45RO/CCR7/CD28/CD27/CD57/CD25/HLA-DR/CTLA4/PD-1
    - NK cells will be assessed using CD16/CD56/CD69.
    - B-cells and dendritic cells will be analyzed using: CD19, CD123, CD11c, CD86, MHC class I and II, CD70, and CD54.
    - MDSC will be assessed using: CD11b, CD 14, CD33.

- Next generation sequencing of the T-cell receptor-β locus in genomic DNA from sorted CD4+ and CD8+ T cell subsets from blood samples using the TRB ImmunoSeq kit (Adaptive Biotechnologies).
- Tumor biopsies (of an untreated site) in select patients who consent will be assessed for: cell death, tumor infiltrating lymphocytes, expression of cell surface markers including HLA, PDL1, and undergo multiparameter flow cytometry as well as TCR sequencing.

3 Study Design

3.1. General Design

This is a pilot study to test whether densely ionizing, high relative biological effectiveness (RBE) radiation in the form of neutron radiation, can induce a more robust systemic immune response in combination with ADT plus abiraterone + prednisone, than ADT plus abiraterone + prednisone alone, in patients with advanced CSPC. The long-term goal is to improve the response rate to systemic therapy with addition of focal radiation treatment as a "vaccine". Trial schema is shown below in Figure 5 and study calendar in Table 1. Patients with CSPC about to receive ADT plus abiraterone + prednisone therapy as first-line treatment, and eligible to receive radiation treatment to 1-3 sites of disease, will be enrolled and randomized onto one of 2 arms: 1) ADT plus abiraterone + prednisone alone; or 2) ADT plus abiraterone + prednisone and neutron radiation.

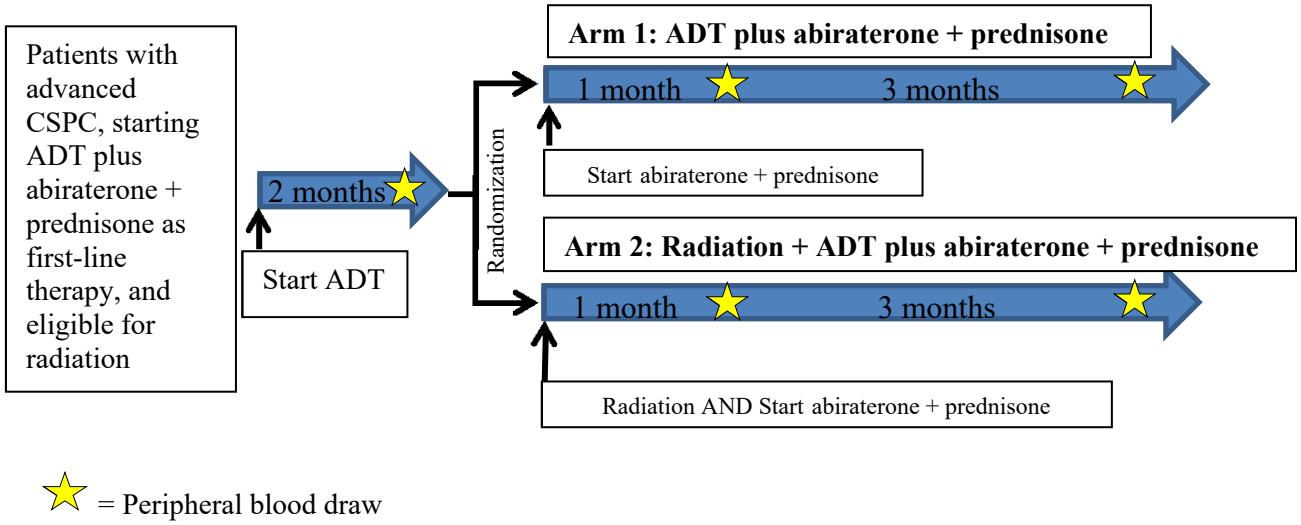

Figure 5. *Clinical trial schema.*

Table 1. Study Calendar

	2-Months After Start of ADT[4]	1-Month After Start of Abiraterone+ Prednisone[7]	4-Months After Start of Abiraterone+ Prednisone[8]	Every 4-Weeks[9] Until 6-Months After Start of Abiraterone
Informed Consent	X			
Medical History	X	X	X	
Physical Exam	X	X	X	
ECOG Performance Status	X	X	X	
Blood Draw[1]	X[5]	X	X	
Radiation Treatment	X[6]			
Tumor biopsy (optional)[2]	X	X		
PSA	X	X	X	X
CT C/A/P[3]	X			
Survival	X	X	X	X

[1]Blood draw will consist of 9 tubes: 8 yellow top tubes (BD Vacutainer #364606 Acid citrate dextrose additives ACD Solution A) and 1 red top tube (BD Vacutainer #367820 Clot Activator) to collect and store the following: 1) 6 tubes of 0.5 ml serum; 2) 2 tubes of 1.0 ml plasma; 3) 4 tubes of 0.5 ml PBMC; and 4) 6 tubes of 1 ml PMBC.
[2]Tumor biopsy of a non-radiated site will be performed for patients who consent.
[3]CT C/A/P is preferred but MRI or PET/CT also acceptable.
[4]Patients may be enrolled onto the trial up to 2 months after starting ADT, and ADT starts from the first dose of LHRH agonists or antagonists. Patients must be enrolled prior to starting radiation treatment since randomization must occur to determine if patient will receive radiation treatment. All pre-treatment assessments must be performed within 30 days of protocol enrollment, except CT C/A/P which must be within 60 days of protocol enrollment.
[5]First blood draw must take place after at least 7 weeks on ADT and be ≤1week prior to starting radiation or abiraterone.
[6]Radiation treatment can happen between 8-10 weeks after starting ADT and must happen within 1-week of starting abiraterone (could be either before or after).
[7]Acceptable date range is 3-5 weeks.
[8]Acceptable date range is 3-5 months.
[9]Acceptable date range is every 3-6 weeks.

Time points for peripheral blood collection are based on prior studies, including Twyman-Saint Victor *et al.*[15],that show changes in peripheral blood immune cell populations after radiation treatment peak around 2-4 weeks but can persist for months after treatment. Tumor biopsy of a non-radiated site will be performed for patients who consent, pre-radiation treatment and post-radiation treatment (2-4 weeks after radiation).

After completion of radiation treatment, patients will continue to obtain PSA checks every 4 weeks per standard of care until 6 months after start of abiraterone (see study calendar in Table 1), and a research coordinator will collect survival data until that point. Patient may be followed by local physicians and does not have to be seen within the UWMC/SCCA system, but will give permission for outside medical records to be obtained. Patients are considered off-study as defined under section 6.1 Duration of Therapy.

3.2. Eligibility Criteria

A. Inclusion Criteria

- Pathologically proven (either histologic or cytologic) diagnosis of prostate adenocarcinoma with <50% neuroendocrine differentiation or small cell histology
- At least one site of nodal or distant metastatic disease that is measurable by RECIST 1.1 criteria, or a bony metastasis that is evaluable on both CT and bone scan
- No prior orchiectomy.
- No androgen deprivation therapy such as treatment with antiandrogens, LHRH agonists or antagonists for at least one year prior to trial enrollment, and testosterone must be inside normal range prior to trial enrollment if there is prior history of ADT.
- No other systemic anti-cancer therapy, including ADT, for at least 1-year prior to enrollment.
- Prior prostate-directed therapies such as prostatectomy or cryotherapy are allowed.
- Prior radiation treatments are allowed (prostate or metastatic sites) but must have been completed at least 3 months prior to starting ADT for this trial.
- Patients must have normal organ and marrow function as defined below:
    - WBC>3000/mm3
    - ANC>1000/mm3
    - Platelets > 100,000/mm3
    - Creatinine <1.5 institutional ULN or calculated creatinine clearance >30 ml/min
    - AST, ALT, and total bilirubin < 3x institutional ULN (unless patient has documented Gilbert's syndrome)
- No steroids for at least 2 weeks prior to enrollment, and patient must not be expected to require steroids during the study period, other than the typical low dose steroid that is given with abiraterone (typically prednisone or prednisolone at 5 mg twice daily).
- Zubrod Performance Status 0-2
- Age ≥ 18
- Patient must sign study specific informed consent prior to study entry
- Men who are sexually active must use medically acceptable forms of contraception

B. Exclusion Criteria

- Other illnesses with a life expectancy of less than 6 months, including but not limited to unstable angina, symptomatic congestive heart failure, cardiac arrhythmias.
- Psychological or social issues that would prevent patients from informed consent or complying with study requirements.

- Subject has a history of unexplained loss of consciousness or transient ischemic attack within 12 months of treatment start.
- Individuals on active treatment for a different cancer are excluded. Individuals with a history of other malignancies are eligible if they are deemed by the investigator to be at low risk for recurrence of that malignancy.
- Known brain metastasis.
- Known allergies, hypersensitivity, or intolerance to abiraterone or prednisone.
- Prior ADT less than a year, or greater than two months, prior to trial enrollment or prior ADT with testosterone less than normal.
- Must not have a gastrointestinal condition that would interfere with absorption.
- There is a potential drug interaction when abiraterone is concomitantly used with a CYP2D6 substrate narrow therapeutic index (e.g., thioridazine, dextromethorphan), or strong CYP3A4 inhibitors (e.g., atazanavir, erythromycin, indinavir, itraconazole, Ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole) or strong inducers (e.g., carbamazepine, Phenobarbital, phenytoin, rifabutin, rifampin, rifapentine). Caution should be used when patients are on one of these drugs.
- Patients with a history of pituitary or adrenal dysfunction, active or symptomatic viral hepatitis, HIV, or chronic liver disease are not eligible.
- Any chronic medical condition requiring a higher dose of corticosteroid than 10 mg prednisone/prednisolone once daily

4 Study Registration

Subjects will be registered by the FHCRC/UW Study Coordinator and entered into the OnCore Clinical Trial Management System (CTMS). A complete, signed, study consent and HIPAA consent are required for registration.

5 Radiation Therapy

All radiation treatments will be administered at the University of Washington Medical Center. All other appointments (medical oncology visits, labs, scans, etc.) can occur at either UWMC or SCCA. Protocol treatment must begin within 30 days of study enrollment.

5.1. Dose Specifications

Radiation treatments will meet all requirements considered standard clinical care. Although the neutron radiation treatment is the study procedure in this clinical trial, it is routinely given as standard clinical care in patients with metastatic prostate cancer, either for palliation of existing symptoms (such as pain from a bone metastasis), or to prevent development of symptoms from a metastatic lesion (such as preventing future fracture from a bone metastasis), or to potentially improve survival (as seen in the STAMPEDE and SABR-COMET trials discussed in the Introduction section). The exact role of radiation therapy in metastatic prostate cancer is controversial (multiple randomized studies are ongoing) and both arms of this study are acceptable as standard clinical care.

Patients should ideally be treated with 3 fractions of radiation but up to 5 fractions may be allowed if treating radiation oncologist is concerned with toxicity. All radiation treatments must complete within a 2 week period. Up to 3 sites may be treated concurrently. For neutron radiation, 2.7 Neutron Gy x 3 fractions is the goal dose, but may be decreased to 1.5 Gy x 3-5 fractions at discretion of treating radiation oncologist.

5.2. Localization, simulation, and immobilization

- All patients will undergo CT based treatment planning at initial simulation per standard clinical care. Immobilization devices will be used as applicable to treated anatomic region. The CT scan must capture the region of interest as well as surrounding organs at risk (OAR) with sufficient margin for treatment planning. The CT scan should be obtained with a uniform slice thickness of less than or equal to 3 mm throughout. The use of IV contrast is left to the discretion of the treating physician.
- All lesions with potential for respiratory motion should be evaluated by appropriate means including 4D CT scan and/or implanted fiducial marker(s). Respiratory motion management including but not limited to active-breathing control, respiratory gating, and fiducial marker tracking, will be employed for qualifying patients per standard clinical practice.
- Daily image guidance will be employed for target localization.

5.3. Target Volumes

- The gross tumor volume (GTV) is defined as all known gross disease encompassing the selected index lesion as visualized the planning CT scan and aided by additional diagnostic imaging studies (PET/CT or MRI). The use of additional diagnostic imaging studies is dependent on the location of the index lesion and is left to the discretion of the treating physician.
- An internal gross tumor volume (IGTV) is defined for mobile index lesions at the discretion of the treating physician. A 4-D CT scan will be acquired in order to account for the motion of the lesion during treatment. The IGTV will be defined as the union of the visualized index lesion on all gated CT data sets.
- The clinical target volume (CTV) can include a margin of 0-10 mm at the discretion of the treating physician.
- The planning target volume (PTV) will be defined as per standard of care.

5.4. Critical Structures

Organ at risk volume (OAR) is contoured as visualized on the planning CT or MR scan. Applicable OARs will be contoured as clinically appropriate based on site treated.

5.5. Treatment Planning

- Any clinically acceptable, standard of care planning technique may be employed to deliver radiation to the index lesion, including 3D conformal treatment, intensity modulated radiation therapy, and stereotactic radiosurgery. Typically, patients should not be treated with two directly opposed beams due to the volume of tissue receiving high dose radiation, and using at least 3 beams or 2 non-opposed beams is encouraged. All plans are subject to review by the PI. All dose calculations will include corrections for tissue heterogeneities as specified by IROC Houston.
- Dose specifications: at least 95% of the target volume (PTV) is covered by at least 90% of the prescription dose.
- Critical Organ Doses: All standard of care critical organ dose-volume limits will be respected.

5.6. Radiation Quality Assurance Reviews

All patients treated on this protocol will undergo standard review in the Department of Radiation Oncology. At least two physicians will review the patient history, imaging findings, tumor contours, and radiation plan.

5.7. Radiation Toxicity

Toxicity will be graded based on CTCAE 4.0.

5.8. Criteria for Removal/Withdrawal from Treatment

Patients will be withdrawn from treatment if their clinical conditions decline so they are no longer able to tolerate radiation, or are unlikely to clinically benefit from further therapy.

Patients will still receive follow up care per standard of care even if they withdraw from the study. If a subject withdraws consent to participate in the study or aspects of the study, attempts will be made to obtain permission to record at least survival data up to 6 months post-treatment.

6 Drug Therapy

Patients will receive ADT and abiraterone + prednisone per standard of care with medical oncology.

- ADT will be given as LHRH agonists or antagonists (i.e. leuprolide).
- First-generation androgen receptor antagonists such as bicalutamide may be given for up to two months concurrently when the LHRH agonist is started, but must be discontinued after two months to minimize potential immunologic impact on subsequent radiation treatment.
- Abiraterone acetate will be given per standard clinical care, with prednisone.
- Dose adjustments are allowed at the discretion of the treating oncologist after patients have started on treatment.
- No other anti-cancer therapy may be administered during the study.
- Bone protective therapy with zoledronic acid or denosumab is allowed.

Abiraterone + prednisone will be administered for at least 6 months, or until disease progression as defined below, unless treating oncologist feels patient is still deriving a benefit from therapy:

- PSA progression per PCWG3 criteria: ≥ 25% and ≥ 2 ng/mL above the nadir, confirmed by a second value ≥ 3 weeks later
- Radiographic progression based on RECIST 1.1 and PCWG3 criteria
- Symptomatic progression e.g. worsening pain or investigator discretion

6.1. Duration of Therapy

- Disease progression (see above under section 6. Drug Therapy) unless treating oncologist feels patient is still deriving a benefit from therapy.
- Intercurrent illness that prevents further administration of treatment.
- Unacceptable adverse event(s).
- Subject decides to withdraw from the study.
- General or specific changes in the subject's condition render the patient unacceptable for further treatment in the judgment of the investigator.

7 Adverse Event Reporting Requirements

7.1. Adverse Event (AE) Reporting

An AE is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study. Abnormal laboratory values or diagnostic test results constitute AEs only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

AEs of Grade 3 and above (per CTCAE v4.0) will be monitored and recorded in study-specific case report forms (CRFs) in the REDCap system from the time of the first study treatment through 30 days following the end of study treatment, or until the patient receives an alternative anti-cancer therapy, whichever date comes first. AEs related to tumor biopsies that are done solely for research will be monitored, recorded, and reported according to the same standards.

7.2. Expected Toxicities

ADT and abiraterone + prednisone will be given per standard of care and is generally well-tolerated based on phase III data. Full prescribing information for abiraterone can be found at: https://www.zytiga.com/shared/product/zytiga/zytiga-prescribing-information.pdf.
Prescribing information for prednisone can be found at:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202020s000lbl.pdf,
Prescribing information for leuprolide can be found at: http://www.rxabbvie.com/pdf/lupronuro_pi.pdf.

Neutron radiation will also be given per standard of care at palliative dosing, which is also generally well tolerated. Exact toxicity depends on site of treatment and typically includes fatigue and dermatitis in the treatment field.

The grade and severity of the event will be determined using CTCAE v.4.0. The event will be determined to be expected or unexpected. The determination of whether an AE is expected is based on ADT, abiraterone, and prednisone-specific adverse event information per prescribing information, as well as site specific toxicity listed on radiation treatment consent form for patients receiving radiation treatment. Unexpected AEs are those not listed in the agent-specific adverse event information nor radiation consent form. The event will be evaluated for relationship to the medical treatment or procedure. The Investigator should document his/her opinion of the relationship of the event to study medication as follows:

- Unrelated- The adverse event is clearly not related to the investigational agent(s).
- Possible-The adverse event may be related to the investigational agent(s).
- Probable-The adverse event is most likely related to the investigational agent(s).
- Definite- The adverse event is clearly related to the investigational agent(s).

Based on this information, institutional guidelines will be followed regarding whether an adverse event should be reported as an expedited report in addition to the routinely reported clinical data.

8 Data and Safety Monitoring Plan

Oversight for this study will be provided by the Principal Investigator with delegation of appropriate responsibilities to sub-investigators and designated study personnel. They will ensure all entry criteria are met prior to the initiation of the protocol and all study procedures and reporting of adverse events are performed according to the IRB-approved protocol.

8.1. Early Stopping Rules

Early stopping of this trial will be any grade 5 adverse events (AEs) or multiple grade 4 (2 or more) AEs occurring within ≤ 30 days after the end of treatment defined as possibly, probably, or definitely related to radiation treatment (per CTCAE, v.4.0). All AE's grade 3 or higher must be reported to the PI within 24 hours.

8.2. Interim Data Review

All immunologic correlate analysis will be batched. Interim reports will be prepared twice per year until the initial treatment results have been presented or published. In general, the interim reports will contain the following information:

- Patient accrual rate with a projected completion date (while the study is still accruing)
- Total patients accrued
- Frequencies and severity of adverse events
- Compliance rates of treatment delivery

Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCRC Scientific Review Committee (SRC) and the FHCRC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

9 Data Management/Confidentiality

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique patient number to assure subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents.

Subject research files are stored in a secure place (or database). Access is restricted to authorized personnel.

10 Statistical Considerations

10.1. Study Population

This is a pilot study comparing the immunologic response to neutron radiation plus ADT and abiraterone + prednisone versus ADT and abiraterone + prednisone alone. Patients are expected to receive ADT per standard clinical care, and study enrollment is allowed up to two months on ADT. After enrollment, patients are randomized in a 1:1 ratio to two arms to receive either: 1) abiraterone in addition to ADT, or 2) abiraterone+radiation in addition to ADT. There will not be stratification factors. Target accrual is 30 patients receiving treatment per randomization. If a patient enrolls but cannot be randomized or cannot receive treatment per randomization, they will be off study. Data analysis will be per treatment arm.

10.2. Primary Objective

The primary objective will compare the percent change in peripheral blood effector T-cells (CCR7-/CD45RO-) between the ADT+abiraterone arm and the ADT+abiraterone+radiation arm, measured at immediately prior to starting abiraterone, and 1 month after start of abiraterone (which is also about 1 month after radiation treatment in the radiation arm). Percent change in peripheral blood effector T-cells will be calculated by measuring the difference of the percent peripheral blood effector T-cells for each patient between two time points: pre-abiraterone treatment and 1 month post-abiraterone treatment (which is also 1 month post-radiation in the radiation arm). No one has published on the effect of abiraterone on immune cell populations in the peripheral blood. Depending on distribution of the percent change, unpaired two-sample t-test, or Wilcoxon rank-sum test, or log transformation will be used to test the null hypothesis that the percent change in peripheral blood effector T-cells is equal between the two arms.

In order to detect an estimated 10% larger increase in peripheral blood effector T-cells (CCR7-/CD45RO-) in the radiation arm versus the no radiation arm at 1-month post-abiraterone+radiation, to achieve 80% power at two-sided type I error of 5%, while assuming a 10% standard deviation in each patient group, around 15 patients are required per arm. A study by Morse *et al.* described above in section 1 saw approximately 10% increase in peripheral blood effector T-cells (CCR7-/CD45RO-) in response to ADT in patients with prostate cancer at 1-month after starting ADT. Abiraterone further suppresses testosterone levels in addition to ADT and could cause further changes in peripheral blood effector T-cells. Multiple other studies referenced above in section 1 show that an approximately 10% increase in peripheral blood T-cells could be correlated with improved clinical outcomes.

10.3. Secondary Objectives

- To evaluate the rate of patients with undetectable PSA (<0.2 ng/mL) at 6-months after start of abiraterone.
    - This rate will be calculated using Kaplan-Meier estimates and reported for all patients on the trial and for each treatment arm individually. Based on past publications in patients receiving ADT+abiraterone as first-line therapy, the expected rate of undetectable PSA at 6-months is around 95%[2]. This study is not powered to detect a difference between the two arms in this endpoint, and no comparison is planned for this endpoint between the two arms.
- To evaluate the frequency and severity of toxicities as measured by CTCAE 4.0.
    - Descriptive statistics will be used to report the frequency and severity of toxicities for all patients on the trial and for each treatment arm individually.
    - Based on past publications in patients receiving ADT+abiraterone as first-line therapy[2], 99% of patients are expected to experience toxicity of any grade, with approximately 50% of patients experiencing grade 3 or higher toxicity.
    - The most common toxicities of any grade include hot flashes (90%), fatigue (77%), musculoskeletal disorders (72%), impotence (55%), blood count changes (45%), and cardiovascular disorders (43%).

- The most common grade 3 or higher toxicities include endocrine disorders (including hot flashes and impotence) 15%, cardiovascular disorders (10%), musculoskeletal disorders (7%), and hepatic disorders (7%).
- The radiation treatment is expected to cause toxicity related to palliative radiation, which are usually mild and transient. Typical toxicities include grade 2 or less fatigue and dermatitis. Depending on body site treated, nausea, esophagitis, and musculoskeletal pain can also be seen.

10.4. Exploratory Objectives

- Changes in peripheral blood immune cell subpopulations measured via multi-parameter flow cytometry at all three specimen collection time points will be compared between the arms. Percent of each immune cell subpopulation in the peripheral blood will be calculated at three time points for each patient: pre-abiraterone treatment, 1 month post-abiraterone treatment (which is also 1 month post-radiation in the radiation arm), and 4 months post-abiraterone treatment (also 4 months post-radiation in the radiation arm). The percent change from pre-treatment to 1-month post-treatment, and the percent change from pre-treatment to 4-months post-treatment will be calculated for each patient and compared between the two arms. No one has published on the effect of abiraterone on immune cell populations in the peripheral blood. Depending on distribution of the percent change, unpaired two-sample t-test, or Wilcoxon rank-sum test, or log transformation will be used to test the null hypothesis that the percent change in peripheral blood immune cell subpopulations is equal between the two arms.
  - Important T-cell subsets using markers such as: CD3/CD8/CD4/Foxp3/CD45RA/CD45RO/CCR7/CD28/CD27/CD57/CD25/HLA-DR/CTLA4/PD-1
  - NK cells will be assessed using CD16/CD56/CD69.
  - B-cells and dendritic cells will be analyzed using: CD19, CD123, CD11c, CD86, MHC class I and II, CD70, and CD54.
  - MDSC will be assessed using: CD11b, CD 14, CD33.
- Next generation sequencing of the T-cell receptor (TCR)-β locus in genomic DNA from sorted CD4+ and CD8+ T cell subsets from blood samples using the TRB ImmunoSeq kit (Adaptive Biotechnologies). TCR diversity and clonality for each patient will be compared at the three sample collection time points: pre-abiraterone treatment, 1 month post-abiraterone treatment (which is also 1 month post-radiation in the radiation arm), and 4 months post-abiraterone treatment (also 4 months post-radiation in the radiation arm). Hypothesis generating differences will be sought between the two treatment arms. With 15 patients per arm, we do not expect statistically significant differences between the two arms.
- Tumor biopsies (of an untreated site) in select patients who consent will be assessed for: cell death, tumor infiltrating lymphocytes, expression of cell surface markers including HLA, PDL1, and undergo multiparameter flow cytometry as well as TCR sequencing. TCR clonotypes will be compared in tumor biopsies and peripheral blood.

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