COVER PAGE

Study Title: Randomized trial assessing induction of double strand breaks with androgen receptor partial agonist in patients on androgen suppression

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Randomized trial assessing induction of double strand breaks with androgen receptor partial agonist in patients on androgen suppression

Phase: 0

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Location of study: The study will be performed in the Harry and Jeanette Weinberg Building of the Johns Hopkins Oncology Center, 401 North Broadway, Baltimore, Maryland.

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1.0 Background

Although active surveillance for very low-risk prostate cancer has become more prevalent, evidence for the importance of local therapy for patients with intermediate-, high-risk and even oligometastatic disease is growing (1,2). The pivotal role of androgen signaling in prostate cancer progression has long been recognized, and suppression of androgen receptor-mediated effects on prostate cancer remains a pillar of its clinical management. For high-risk, potentially lethal prostate cancers, radiation is the only local treatment modality which has level 1 evidence demonstrating improved survival when used in concert with androgen suppression (2,3). However, in spite of the value of this combined approach, these patients remain at risk of dying from prostate cancer. Local recurrence remains the predominant mode of treatment failure, and strategies to improve the therapeutic index of radiation based therapy of intermediate and high risk prostate cancer are critically needed (3,4).

While studying androgen receptor (AR) transcriptional signaling in prostate cancer, our group unexpectedly found that stimulating AR transcriptional programs in androgen-deprived prostate cancer cells using AR agonists such as testosterone and dihydrotestosterone (DHT) leads to production of numerous, transient DNA double strand breaks (DSB) that require DNA repair machinery for resolution [4–6]. These double strand breaks were mediated by the class II topoisomerase TOP2B, which acted as a transcriptional co-activator with the androgen receptor. Although all of the mechanistic causes and roles of these DSB in AR signaling are still unclear and are the subject of intense ongoing research in the group, the finding that AR stimulation could lead to TOP2B mediated DSB was highly reproducible and robust and presented a potential Achilles Heel of AR signaling in prostate cancer cells. In preclinical studies, the DSB produced by treatment of cells with a pulse of DHT led to significant selective sensitization of AR-positive cells to treatment with ionizing radiation *in vitro* and *in vivo* [6].

Unfortunately, although these preclinical studies appeared very promising, the prospect of overt AR stimulation, even transiently, in treatment of localized prostate cancer (where conventional androgen deprivation plus IR therapy can be curative in many cases) raised many concerns for further clinical translation given the dependence of prostate cancers on AR signaling; thus, alternative strategies to overt AR stimulation were considered.

In particular, we hypothesized that some AR antagonists may be able to partially stimulate the AR and induce TOP2B-mediated DSB without allowing activation of AR mediated growth stimulation and transcriptional programs. Remarkably, in new preliminary data, among a series of tested AR antagonists, hydroxy-flutamide (FLU) allowed nuclear translocation of AR, and stimulated genome wide TOP2B-mediated DSB. In fact, when exposed to 10 uM FLU, a clinically achievable concentration, androgen-deprived, AR-positive, prostate cancer cells accumulated DSB, measured by neutral comet assay, at levels equivalent to irradiating them with 0.5 to 2 Gy of ionizing radiation. The level of breaks was also equivalent to that achieved by saturating levels of the AR agonist DHT. Androgen-deprived PCa cell lines also displayed increased γH2A.x foci, a widely accepted biomarker of DSB. Interestingly, the second generation, highly potent AR antagonist enzalutamide did not induce DSB in prostate cancer cells. Immunofluorescent staining of the androgen receptor (AR) demonstrated robust nuclear translocation in androgen-deprived cells following exposure to a wide range of doses of FLU but not to Enzalutamide, providing some explanation of the differing DSB inducing ability of these AR antagonists (see **Fig 1** below).

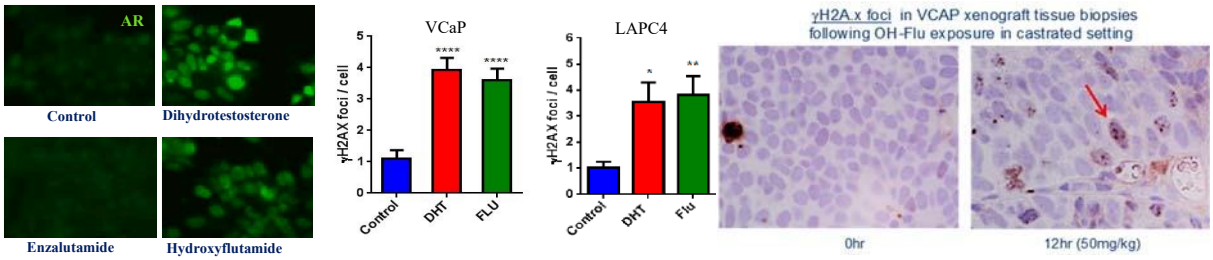
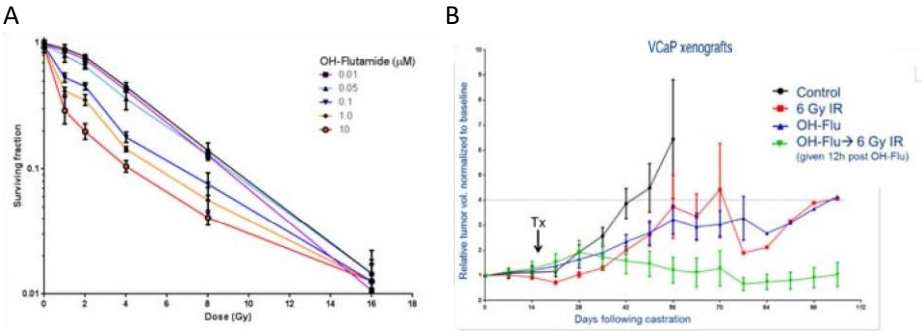


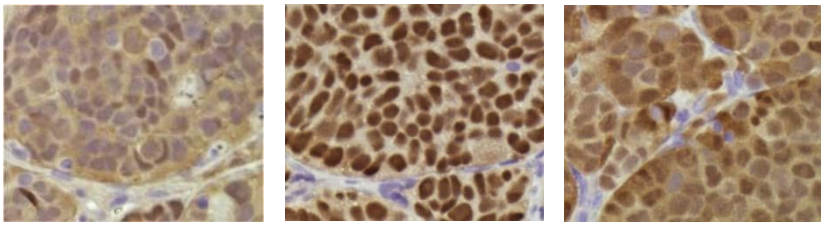
Fig. 1 The AR antagonist FLU, but not Enzalutamide, allows nuclear localization of AR (left). Flutamide, like DHT, stimulates DSB in AR+ Pca cells *in vitro* (middle) and *in vivo* (right).

Moreover, RNAi-mediated knockdown of either AR- or topoisomerase 2 beta (TOP2B) prevented DSBs resulting from FLU exposure, which is consistent with mechanistic observations following stimulation with DHT [6]. Importantly, although LNCaP cells have a mutation in the AR increasing its activity, we also observed DSB formation in VCaP, CWR22Rv1, and LAPC4 cells as well, suggesting that PCa cells with wild-type AR will respond similarly. γH2AX foci increased over 6h exposure to DHT or FLU, yet this increase in γH2AX could be prevented by co-incubation with the antioxidant *N*-acetyl-*L*-cysteine (NAC, 5mM), suggesting that reactive oxygen species (ROS) contribute to the TOP2B-mediated DNA damage following AR signaling. To test the possibility that AR-mediated DSBs may radiosensitize AR-expressing PCa cells, DHT and AR-antagonists were used to induce DSBs in androgen-deprived LNCaP cells, followed by exposure to IR. Interestingly, a pulse of DHT or FLU significantly and synergistically enhanced the radiosensitivity of AR-positive VCaP prostate cancer cells (**Fig 2A**). Stimulation of castrated nude mice implanted with prostate cancer xenografts with a pulse of FLU followed by treatment with ionizing radiation also led to significant growth inhibition of the xenografts compared to mice treated with FLU alone or ionizing radiation alone, suggesting therapeutic benefit of the paradigm in vivo (**Fig 2B**).

Fig. 2. (A) Profound synergy of FLU plus IR in reducing clonogenic survival of VCaP cells in vitro. (B) Combination of a pulse of FLU followed by 6 Gy IR inhibits VCaP xenograft growth significantly greater than either agent alone.



Importantly, in these in vivo studies, immunohistochemical examination of biopsies from xenograft tissue displayed increased γH2AX foci, which increased from 0-12 hours and returned to basal levels by 24 hours. Similarly, AR was observed to be highly nuclear in localization under similar time-points (**Fig 3**).



These observations, if confirmed in humans, are potentially readily translatable to clinical practice. Such an effect would enhance the therapeutic index given its high specificity to AR-expressing tissues, and could be exploited on a repeated basis over a course of fractionated RT by dosing FLU at spaced intervals.

Fig. 3. AR in VCaP xenograft tissue biopsies following OH-Flu exposure in castrated setting

The primary goal of this study is to carry out a first-in-man pharmacodynamics clinical trial to test whether a pulse of FLU can induce DSB in men undergoing androgen suppression as part of their treatment strategy for localized prostate cancer.

2.0 Study Objectives

Primary objective:

To confirm DNA double strand breaks occur in prostate cancer tissue following pulse-dose Flutamide administration in patients who are androgen suppressed, as compared to control patients receiving placebo.

Exploratory objective:

To explore the feasibility of detecting cancer-specific alterations in DNA methylation and structure within urine and plasma, and the kinetics for these measures when compared with PSA values and clinical response. We will examine whether prostate cancer specific DNA methylation alterations at a panel of frequently hypermethylated loci (e.g. GSTP1, PTGS2, MDR1, APC, RARbeta, gene promoters) can be used as a DNA based biomarker for tracking therapeutic response.

3.0 Study Population

The target population will be patients with a diagnosis of adenocarcinoma of the prostate who are seen in consultation at the Department of Radiation Oncology and Molecular Radiation Sciences. Approximately 400 patients per year with the diagnosis of prostate cancer are seen in the Department of Radiation Oncology. Within this group of patients, approximately 50% are eligible for brachytherapy based on having intact prostate (not post-prostatectomy), gland size, relative absence of lower urinary tract obstructive symptoms, and no prior transurethral resection of prostate (TURP). Of this subset, approximately 60% will receive androgen suppression with brachytherapy due to intermediate- or high-risk disease. Approximately 80% of patients offered brachytherapy at consultation decide to receive their treatment at Johns Hopkins. Overall, we currently perform 4 brachytherapy procedures per month on average, for a yearly total of approximately 50 cases.

3.1 Eligibility criteria

3.1.1 Inclusion criteria

- Histologically confirmed, localized (M0) adenocarcinoma of the prostate
- Clinical stages T1c – T3b, Mx or M0
- At least one biopsy core with Gleason 7 or higher disease
- The patient has decided to undergo brachytherapy plus androgen suppression as treatment modality for his prostate cancer (with or without supplemental external beam radiation)

- Suitable volume of disease for biopsy, defined as one or more of the following:
    - MRI scan of the pelvis with ≥ 1 identifiable lesion as being PI-RADS 4 or 5, plus minimum of at least 0.5cm in any dimension

      OR
    - Clinically palpable disease corresponding to (ipsilateral to) any involved core on biopsy

- Signed study-specific consent form prior to registration

3.1.2 Exclusion criteria

- Known hypersensitivity or allergic response to Flutamide
- Severe hepatic impairment (serum ALT level >2x normal or serum AST level >2x normal)
- Pre-existing diagnosis of hypogonadism (serum total testosterone < 150 ng/dL) prior to starting hormone therapy for prostate cancer, or treatment with testosterone supplementation therapy within 12 months prior to enrollment
- Major medical or psychiatric illness which, in the investigator's opinion, would prevent completion of treatment and would interfere with follow up.

4.0 Protocol Design

This will be a prospective, single-center, two-phase study to assess the efficacy of single pulsed-dose Flutamide in creating DSBs in prostate cancer within patients receiving central androgen suppression and brachytherapy. The initial phase is a 6-patient single-arm run-in phase to test feasibility and rule out futility of the intervention. All patients within the run-in phase will receive 250mg Flutamide prior to brachytherapy and prostatic biopsy. Biostatistician will be consulted upon completion of run-in phase. If futility is ruled out (>1 patients with DSBs on biopsy), then accrual will continue in the randomized, double-blind phase. Subjects will be randomized in a 2:1 ratio (12:6 patients), for total of 18 patients randomized receiving Flutamide and 6 patients receiving placebo. Thus, total evaluable patients including both phases (non-randomized/run-in plus randomized) will be 24 patients, with 30 enrolled to ensure minimum of 24 evaluable.

4.1 Subject Identification

Patient confidentiality will be maintained in accordance with Health Information Portability and Accountability Act (HIPAA) guidelines. All participants must sign an informed consent that will describe the objectives of the study and potential risks. All patient data reported on the case reports forms will be

identified by the patient's initials and study code number only. Patients shall not be identified by name. This should serve to protect the confidentiality of subjects enrolled on the trial. Clinical data and records for all subjects studied including history and physical findings, laboratory data, and results of interventions are to be maintained by the investigators in a secure, locked location. Computerized data will require password authorization(s) for access.

4.2 Description of the Recruitment Process

Potential subjects will be identified at the time of consultation in the Department of Radiation Oncology or in the Precision Medicine Center of Excellence for prostate cancer.

4.3 Description of the Informed Consent Process

Only the principal investigator or those listed as co-investigators will perform the informed consent interview. The informed consent interview will take place before the patient is to be treated to ensure that the patient has adequate time to discuss the research project with family, friends, and/or other Health Care providers. During the informed consent interview, the interviewer (investigator) will take as much time as needed to ensure that the potential subject understands the research project and also clearly understands that he does not have to participate in this project to receive his cancer treatment at Johns Hopkins.

4.4 Subject Assignment

We will enroll 30 patients onto the trial in order to ensure at least 24 evaluable patients.

This will be a two-arm study with randomization. We will apply 2:1 randomization to treatment (Flu) vs placebo arm available and to confirm Flu-induced increase in γH2AX foci vs control. All patients would receive standard androgen suppression and brachytherapy in accordance with their disease parameters and risk stratification.

The measurement of Flu may be accordingly transformed to ensure the necessary distributional and model assumptions are met. Analyses for all secondary endpoints will be exploratory in nature, and specified in detailed in the study protocol and statistical analysis plans.

4.5 Clinical Assessments / Study Calendar A

In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants' risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

A synopsis of the study procedures is shown below:

			PRE-BIOPSY / IMPLANT			BIOPSY / IMPLANT DAY [A]		POST-BIOPSY / IMPLANT	
Parameter	Pre-Study	Pre-androgen suppression	-180 to 0 DAYS [G]	-10 to 0 DAYS	-12 TO -6 HOURS	PRIOR TO IMPLANT	TRANSPERINEAL BIOPSY OF INVOLVED REGIONS (S) OF PROSTATE (DEFINED ON MRI OR BY PALPATION)	4 weeks (+/-7 days)	6 months (+/-45 days)
H&P, LABS (CMP)	X								
Study Eligibility	X								
MRI of prostate	X								
Androgen suppression w/ GnRH analogue	X [G]		X [G]						
Study Drug Admin (250mg vs Placebo)					X				
LABS (PSA / Testosterone)	X		X [C, H]					X	X
Plasma for cell free DNA [B]		X [D]		X [C, D]				X [D]	X [D]
Urine (50 mL)		X [D]		X [C, D]				X [D]	X [D]
Plasma for hydroxyflutamide [F]				X [E]		X			

[A] Day of biopsy and brachytherapy implant procedure.

[B] Streck Tubes (10 mL) x 4

[C] Samples may be obtained either pre- or post-flutamide ingestion, but prior to biopsy/implant

[D] These items are separate (optional) consent for exploratory analysis.

[E] -10 to 0 Day Timepoint for non-randomized cohort (n=6) only; sample taken prior to hydroxyflutamide ingestion

[F] Heparin/Green Top Tubes (4 mL) x 1

[G] Patient may be enrolled already on GnRH analogue, or receive after enrollment. Brachytherapy to be performed anytime within 180 days from initiation of androgen suppression provided the patient's testosterone level is < 50 ng/dl.

[H] PSA and Testosterone should be done at least 30 days after Initiation of Androgen Suppression and within 180 days prior to brachytherapy.

***** ADDITIONAL IMPORTANT NOTES ON NEXT PAGE OF PROTOCOL *****

Important notes:

- Timing of brachytherapy
    - Brachytherapy to be performed anytime within 180 days from initiation of androgen suppression provided the patient's testosterone level is < 50 ng/dl.
- Patient will be **off-protocol if serum testosterone at 0-7 days prior to implant is above castrate level (>50 ng/dL or >1.7 nmol/L)**
- Approximately **1/3 of each subject's total biopsy material will be frozen** for future laser capture RNA sequencing

4.6 Data to be collected

The data that will be collected for this trial will be the results and/or findings of the procedures and assessments that are outlined in 4.5 and 4.6. Results/findings will also be abstracted from the source documents and records (e.g. clinic notes, radiology reports, lab reports, and recorded onto either study specific case report forms or within electronic database housed on the secure Radiation Oncology network drive. The data manager assigned to this trial will be responsible for the data collection with regard to clinical elements (PSA, etc.). The principal investigator or coinvestigators and/or assistants may also be responsible for collection and analysis of dosimetric and imaging data. Both data managers and/or investigators will have access to patient medical records and will record medical information from the medical record onto the relevant case report forms or database. The principal investigator will review the data on a quarterly basis for accuracy.

5.0 Research Interventions

Patients who will be offered this protocol are those who are currently offered brachytherapy as standard treatment at our institution.

5.1 Pre-implant Flutamide vs placebo dosing

Patients will receive either Flutamide single 250mg dose or placebo, taken orally 6-12 hours prior to the brachytherapy.

5.2 Plasma, urine and serum sampling for analyses

5.2.1 Plasma assay for 2-hydroxyflutamide

2-hydroxyflutamide levels will be assayed both pre- and post-flutamide dosing in the initial non-randomized cohort. For the randomized cohort, only post-flutamide levels of 2-hydroxyflutamide will be assayed.

Blood samples (~4 ml) will be drawn into heparinized test tubes 0-10 days before drug administration (non-randomized initial cohort only), and also at 6-12 hours post-ingestion of Flutamide. Blood samples are to be immediately placed on ice after collection and centrifuged at 1300g for 10 minutes at 4 degrees C, and the plasma fraction to be separated and stored in polypropylene tubes at temp of -70 degrees C until analysis.

For analysis, specimens will be transferred to Analytical Pharmacology Core Laboratory and analyzed based on method described by Niopa et al. [7]

5.2.2 Serum and urine exploratory analyses

For purposes of exploratory analyses, serum and urine samples will be collected pre-androgen suppression, post-androgen suppression, 1-month post-implant, and 6-month follow-up times. Patients will be asked to sign consent specifically for these samples, otherwise they have the choice to opt out. Given that these analyses do not affect the primary endpoint, patients opting out of the exploratory serum and urine analyses will not adversely affect the study endpoint.

For each urine and plasma sample, the qMBD-seq approach (Yegnasubramanian 2011) will be used to determine the MI and SR scores to assess levels of cancer specific DNA methylation and structural alterations, and the kinetics for these measures will be determined at the timepoints collected and compared with PSA values. As a part of the exploratory analysis consent, patients will also be consented to allow access to any subsequent biopsy and corresponding PSA that may be obtained through standard clinical care, to look at the relationship of these markers.

This analysis will help us to explore the feasibility of detecting cancer-specific alterations in men with prostate cancer. We will also examine whether prostate cancer specific DNA methylation alterations at a panel of frequently hypermethylated loci (e.g. GSTP1, PTGS2, MDR1, APC, RARbeta, gene promoters) can be used as a DNA based biomarker for tracking therapeutic response using our highly sensitive COMPARE-MS DNA methylation assay.

Blood collection

In most cases, blood samples will be drawn from patients scheduled to have venipuncture for routine clinical purposes. In some cases, when this is not possible, blood draws will occur at times other than those needed for routine clinical care. Generally, blood draws for research purposes will be 4 tablespoons of blood (amounts to 4 x 10mL tubes).

Blood Processing. Generally, the processing and storage of blood samples will involve the following: blood will be drawn into 4 Streck tubes for collection and stored as plasma, serum, white blood cells or whole blood. Serum will be separated from other cellular components by centrifugation, allocated into tubes, catalogued, and frozen at –70 to -80° C or viably in liquid nitrogen freezers. Samples may be processed for DNA, RNA, and/or protein. Specimens will be barcoded for inventory management and for tracking clinical study usage.

Urine collection and processing

Urine collected from patients is known to contain nucleic acid material that could serve as a biomarker for cancer. In addition, urine studies may involve purification of proteins and/or cells. Urine is self-collected. Sterile cups will be provided by the clinic.

In Dr. Yegnasubramanian's laboratory, urine preservative (Norgen BioTEK Corp, Cat # 18124) will be added to the urine and stored at room temperature or 4 degrees C until further processing.

Purification of Nucleic Acid from biospecimens (urine/plasma):

DNA purification from plasma and urine will be accomplished using a commercially available Qiagen virus vaccum kit. (QiaVirus vaccum kit, Cat # 57714). Starting with 1 ml of plasma or 10 ml of urine prepared as detailed above (thawed @ RT). The procedure for plasma is described below. Similar column purification method will be used for urine with the notable difference of a larger input volume due to relative scarcity of nucleic acid. Briefly, we will transfer 500 µl plasma to two separate 2 ml tubes, add 40 µl Proteinase K and

mix by vortexing, add 5.6 µl 1µg/µl RNA carrier to 500 µl and mix by vortexing. The specimen will be incubated at 1 h @ 60°C in heating block, then cooled on ice before adding 600 µl EtOH, mixed throughly by vortexing and incubated 5 min at room temperature. The sample (lysate) will be transferred to a vacuum purification column w/extension tube placed on a vacuum manifold. The column will be washed to purify the nucleic acids before the eluate drawn off the column in nuclease free molecular biology grade water (~100ul). *qMBD-seq:* The qMBD-seq approach involves division of DNA extracted from each plasma and urine sample into two fractions: a total input fraction, from which structural alterations can be measured, and a methyl-binding domain (MBD)-enriched methylated fraction, from which DNA methylation changes can be measured (see Figure 1). Briefly, each DNA sample from urine or plasma will be spiked in with a fully methylated lambda-phage DNA internal control, fragmented, and ligated to barcoded sequencing adaptors. Half the sample will be set aside as the total input, and the other half will be subjected to enrichment of methylated DNA using MBD-conjugated magnetic beads as we have described previously[8,9–11]. The resulting enriched methylated fraction and the total input fraction will then be amplified, and the resulting libraries will be subjected to 50x50 bp paired end next generation sequencing. In each fraction, the number of reads mapping to each of 350 regions that we have previously shown to be highly recurrently (>80% of prostate cancers), and stably (maintained through the disease course and across metastatic dissemination) methylated in a prostate cancer specific manner (see Figure 2) will be determined. The number of reads mapping to the fully methylated spiked-in internal quantitation standard will also be determined. The MI score will be defined as: $MI = (H_E/H_T)/(S_E/S_T)$, where H_E and H_T are the number of reads mapping to the prostate cancer specific hypermethylated regions in the enriched and total input fractions respectively, and S_E and S_T are the number of reads mapping to the spiked-in internal standard in the enriched and total input fractions respectively. Conceptually, this MI score is related to the amount of methylated DNA derived from the 350 regions that we have previously identified to be highly frequently and stably hypermethylated in human prostate cancer but not in any normal tissues assessed (including normal prostate, lymph nodes, spleen, liver, kidney, and blood). This approach essentially allows massively parallel assessment of all of these regions in a single assay. If in the future, we needed to include other regions or subtract regions, this can simply be done informatically without any changes needed to the actual assay. Furthermore, the number of paired end reads showing evidence of rearrangements (each sequence from a paired-end fragment mapping to discontiguous portions of the genome), determined as described previously[12,13], in the total input fraction normalized per million overall reads (SR score) will provide a parallel measure of the amount of DNA containing genomic rearrangements. By measuring these alterations using an unbiased genome-wide approach, we can be less limited by stochastic factors that can prevent assessment of a single locus. Furthermore, by measuring both the DNA methylation and structural alterations, we are less prone to technical issues that may limit measurement of any one of these alteration classes. Finally, assessment of both urine and plasma, both of which can be readily and non-invasively obtained, will allow a higher chance of detecting these alterations. The accuracy and precision of each type of analysis will be explored using prostate cancer cell line DNA spiked in a dilution series into normal plasma and urine.

A participant's care will not be altered if they choose to opt out of the exploratory plasma/urine analysis portion of the study.

5.3 Biopsy and implant procedure

In the operating room, patient will be anesthetized per clinical routine for brachytherapy, and placed in dorsal lithotomy position. A transrectal ultrasound (TRUS) probe will be placed into the rectum for prostate visualization. The perineum will be prepped in sterile fashion.

TRUS images will be captured and fused with the MRI to identify the regions of prostate cancer involvement to be targeted. Transperineal biopsy will be carried out under TRUS guidance, with ≥4 cores targeted per MRI-defined region of involvement / clinically palpable region. The number of biopsy cores taken is not to exceed 12 (twelve) cores.

Following completion of the biopsy portion of the procedure, patient will undergo brachytherapy per usual clinical routine.

Biopsy samples will be processed for paraffin embedding and pathologic confirmation of tumor tissue presence (handled and stored by DeMarzo laboratory). Analyses for DSBs will be performed using assays for TUNEL, 53bp1, and gamma h2ax. Approximately 1/3 of tissue will be frozen and set aside for future potential laser dissection capture for RNA sequencing (handled and stored in Yegnasubramanian laboratory).

Staff from DeMarzo laboratory will arrange collection and archiving of diagnostic material. This will consist of the slides and/or paraffin blocks from the biopsies (both pretreatment and postflutamide/pre-brachytherapy).

Central pathology review will be performed for patients who have agreed / opted to allow us access to any post-treatment biopsies, if performed in routine clinical care, and retrospective review of baseline diagnostic pathologies. Formalin fixed biopsy tissue may be used for correlative DNA methylation analysis.

5.4 Follow-up evaluation

Patients will be assessed per routine clinical practice at 1-month and 6 months post-implant, at which time serum and urine specimens will be collected for exploratory analyses. They will receive considered off trial following the 6-9 month follow-up visit.

6.0 Data Analysis and Statistical Considerations

6.1 Study Design and Endpoints

This pilot study is to preliminarily evaluate if DNA double strand breaks occur in prostate cancer tissue following pulse-dose Flutamide administration in patients who are androgen suppressed. It consists of two parts, a run-in part and randomization part. The run-in part is to preliminarily determine feasibility, i.e., if DNA double strand breaks can be observed, defined as if there are greater than or equal to 5% of prostate cancer cells having gammaH2AX foci. The randomization part aims to better characterize the estimation precision of proportion of prostate cancer cells having gammaH2AX foc with and without Flutamide. During the randomization part, we will continue to monitor the feasibility of observing DNA double strand breaks (see Section 6.3 for details).

6.2 Sample Size Justification and Accrual

This is a pilot trial, and will be utilized to design a larger study to establish measures for such parameters with a reasonable level of precision. We will enroll 30 patients onto the trial, with goal of at least 24 evaluable patients accrued.

In the run-in phase, a total of 6 evaluable patients will be accrued to receive Flutamide administration. We will use a Bayesian monitoring rule to determine if the study is feasible. Specifically, we would consider it is "infeasible" if the probability of not observing greater than or equal to 5% of prostate cancer cells having gammaH2AX foci is more than 50%. Based on the Bayesian monitoring rule in Section 6.3, if there is less than or equal to 1 out of 6 cases with detectable DNA double strand breaks (i.e., greater than or equal to 5% of prostate cancer cells having gammaH2AX foci on patient level), the study will be considered as infeasible and will not proceed into the randomization part.

If the study proceeds into the randomization part, a total of 18 evaluable patients will be randomized in a 2:1 ratio to either receive Flutamide (12 patients) or not (6 patients). A total of 18 patients treated with Flutamide (6 from run-in and 12 from randomization) will produce a two-sided 90% confidence interval with a width ranging from 0.35 to 0.42 (if the true proportion of prostate cancer cells having gammaH2AX foci is 30%-80%). A total of 6 patients in control will produce a two-sided 90% confidence interval with a width approximately less than 0.5 (if the true proportion of prostate cancer cells having gammaH2AX foci is less than 10%).

All eligible patients will be offered enrollment on the study, and we estimate a 20% dropout and nonevaluable rate. Patients who drop out prior to the biopsy/brachytherapy procedure will be replaced; patients who drop out after biopsy/brachytherapy will not be replaced. In order to ensure at least 24 evaluable patients are available, we plan to enroll ~30 patients onto this trial. Given the minimal addition to standard therapy and effort requirement from patient, we expect to accrue at least 2-3 patients/month, and the full accrual will be completed in 2 years.

6.3 Early Stopping for Feasibility

The feasibility of detecting DNA double strand breaks will be based on the proportion of patients not observing greater than or equal to 5% of prostate cancer cells having gammaH2AX foci. We consider it is "infeasible" if the probability of not observing detectable DNA double strand breaks (detectable if greater than or equal to 5% of prostate cancer cells having gammaH2AX foci) is more than 50% and with more than 70% posterior probability. We assume there will only be a smaller proportion of cases in which DNA double strand breaks cannot be observed, e.g., on average only 10% of patients will not have detectable DNA double strand breaks and there will be about 16% chance that the risk will be 20% or more. This corresponds to a Beta(0.1,0.9) prior distribution. Table 6.3.1 summarizes the continuous stopping rule for the 18 evaluable patients treated with Flutamide. The feasibility stopping rule calls for the study to be stopped and will not proceed into the randomization part if there are less than or equal to 1 patients with detectable DNA double strand within the first 6 patients (in the run-in part). If the trial enters randomization part, the feasibility stopping rule calls for accrual suspension if the number of patients with detectable DNA double strand breaks is too low (equivalently, the number of patients without detectable DNA double strand breaks is too high).

Table 6.3.1 Stopping rule for feasibility

# patients without detectable DNA double strand breaks	5	6	7	8	9	10	11
Out of total # evaluable patients	6-7	8-9	11	12-13	14-15	16-17	18

Table 6.3.2 summarizes the operating characteristics based on 5,000 simulations with 18 evaluable patients in terms of how frequent the study would stop based on the stopping rule under different hypothetical feasibility rates, as well as the average sample sizes.

Table 6.3.2 Operating characteristics of the stopping rule for feasibility

Underlying proportion without detectable DNA double strand breaks	0.4	0.5	0.6	0.7	0.8
% of time study stops	19.3%	44%	74.8%	92.4%	99.4%
Expected sample size	16.3	14.1	11.1	8.7	6.9

6.4 Gender and Minority Compliance

Minorities of all races and ethnic groups are eligible for this study and will be encouraged to participate. In conformance with the National Institute of Health Revitalization Act of 1993 with regard to inclusion of women and minority in clinical research, we have also considered the possible interaction between race and treatments. Based on the current demographic trends in our center, we project that 24% of men in this study will be nonwhite. Women do not develop prostate cancer and therefore will not be enrolled.

7.0 Risks/Benefits Assessment

Patients enrolled onto this trial would undergo brachytherapy under anesthesia whether or not they participate in this trial. Therefore, the risks that are associated with the brachytherapy are not risks associated with the research. Similarly, we will only enroll patients who are undergoing androgen suppression as standard of care based on disease characteristics. Furthermore, patients would undergo the standard pre-treatment and post-treatment assessments/studies that are outlined in section 4.7 whether or not they participate in this trial.

Transperineal biopsy under anesthesia – Patient will undergo partial-gland transperineal biopsies as a result of enrollment on this trial. The risks of these biopsies is similar to that of standard prostate biopsies but with less chance of infection or sepsis due to not accessing through the rectum, as well as only targeting regions of known involvement. For comparative purposes, patients who undergo active surveillance have yearly biopsies performed for up to several years in succession until progression, therefore 1 partial-gland biopsy associated with this protocol is relatively modest.

Risk of Flutamide – Flutamide is an FDA approved drug with indication for use in patients with prostate cancer. Since we are only giving a single 250mg dose of Flutamide (compared to standard daily 250mg TID dosing for 4 months or more), it is a very low likelihood that patients will experience any long-lasting toxicity. It is theoretically possible that patients could have an acute adverse reaction, although this is expected to be rare.

Risk of blood draw

Patients will undergo blood sampling prior to the biopsy for pharmacokinetic analysis; patients who opt to participate in the exploratory endpoints will have additional blood draws. There could be pain or unexpected excessive bleeding and/or swelling related to the blood draw(s).

7.1 Psychological Risks - There are no foreseeable psychological risks associated with this protocol.

7.2 Social Risks – There are no foreseeable social risks associated with this protocol

7.3 Legal Risks – There are no legal risks associated with this protocol

7.4 Economic Risks – There are no economic risks associated with this protocol. Any costs that are not part of the patient's standard of care for brachytherapy and standard follow-up will be paid for by research funds.

7.5 Privacy/Confidentiality Risks – All research personnel involved in this trial will comply with HIPAA regulations. Because the data that is collected will contain personally identifiable information the following measures will be taken to minimize the risk of breaching patient confidentiality:

- Patients' clinical data (hard and soft copies) will be stored in locked confidential files in the Radiation oncology protocol office at Johns Hopkins east Baltimore campus, and on encrypted, restricted-access servers within the Radiation Oncology domain.
- Only the study personnel, the Johns Hopkins Clinical Research Office, the Office of Human Research Protection (OHRP), the Johns Hopkins Institutional Review Board, and other regulatory agencies will have access to the study files. The secured Clinical data includes: case report forms, source documentation, signed consent forms, and any material containing patient identifying information.
- This data will be securely stored in the Radiation Oncology protocol office at Johns Hopkins as long as required by governmental regulations.
- All of the agencies noted above who have access to study documents and patient identifier codes are to keep the identity of all subjects on this trial confidential. No information by which patients can be identified will be published.

7. 6 Benefits – There is no expectation that patients will benefit from their participation in this study. There is the potential benefit that through participation subjects in the Flutamide arm will receive treatment that is more effective against their prostate cancer.

7.7 Payments and Compensation – Patients will not be paid or compensated for their participation in this trial.

8.0 Reporting of Serious or Unexpected Adverse Events

This is a research trial that is studying the feasibility and practicability of a new treatment planning system for patients who undergo brachytherapy. There are no expected adverse events associated with this trial. However, the research team will be conducting post-follow-ups on all patients enrolled onto this trial as outlined in section 4.7 and will report any adverse events that are either unexpected or are serious.

"CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0."

8.1 The following guidelines will be followed for reporting serious or unexpected adverse events:

a). All **fatal** events, both **anticipated and unanticipated**, must be reported to the JHM IRB within three (3) working days after the PI learns of the event, whether or not the PI believes the event to be related to the study. All other events, which are both **serious** and **unanticipated**, must be reported to the JHMI IRB within three (3) working days after the PI learns of the event. Events which are **serious** but **anticipated**, should be reported as part of the continuing review application. If any of these Serious Adverse Events requires a change to the protocol or consent form, the PI must make those changes promptly and submit the revised documents to the JHM IRB.

b) **Important** Adverse Events that are **unanticipated** must be reported to the JHM IRB within (10) ten working days. If the Important Adverse Event requires changes to the protocol or consent form, the PI must make those changes promptly and submit the revised documents to the JHM IRB.

C) All other **unanticipated** Adverse Events or changes to the protocol and consent form must be reported to the JHM IRB as follows:

1. If the event affects the risk/benefit ratio for study subjects, the reporting requirement is *promptly*.

2. If the event does not affect the risk/benefit ratio for study subjects, the reporting requirement is *at least annually* through the continuing review application.

8.2 Definitions

Serious Adverse Event: means an event that is

- fatal
- life-threatening
- persistent or significantly disabling or incapacitating
- inpatient hospitalization or prolongation of hospitalization
- congenital anomaly or defect and/or
- a significant medical incident (considered to be a serious study related event because, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.)

Important Adverse Event: means an event, although not a Serious Adverse Event, which still presents an undesirable occurrence that interferes with the subject's usual activities and may be persistent or require treatment. (For example, serious rash, cough, or fever.)

Unanticipated Adverse Event: means an event that results from a study intervention and was not expected or anticipated from prior experience. This includes expected events that occur with greater frequency or severity than predicted from prior experience.

8.3 Following a Study Related Adverse Event

If a patient experiences an adverse event while on study, the following steps will be taken:

1) Establish the cause and severity of the adverse event and determine if said event is related to study participation.
2) Principal Investigator will decide what treatment(s), if any, is/are required.
3) Depending on the type and severity of adverse event, an appropriate follow-up schedule will be constructed which will allow for determination of event outcome.
4) Patient will be followed by principal investigator until the adverse event has resolved.

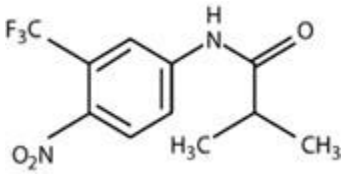
9.0 Description of the Drug

Flutamide is approved by the US Food and Drug Administration for use in patients with (Jewett) stage B2-C prostate cancer (i.e. AJCC stage T1c – T3b), in conjunction with androgen deprivation and radiation therapy.

Flutamide Description

Flutamide capsules contain Flutamide, an acetanilid, nonsteroidal, orally active antiandrogen having the chemical name, α,α,α-trifluoro-2-methyl-4'-nitrom-propionotoluidide.

Each capsule contains 125 mg Flutamide. The compound is a buff to yellow powder with a molecular weight of 276.22 and the following structural formula:



C11H11F3N2O3

In addition, each capsule contains the following inactive ingredients: corn starch, lactose monohydrate, magnesium stearate, povidone, and sodium lauryl sulfate. Gelatin capsule shells may contain gelatin, silicon dioxide, sodium lauryl sulfate, titanium dioxide, FDA/E172 Red Iron Oxide, FDA/E172 Yellow Iron Oxide, and black ink containing pharmaceutical glaze (modified) in SD-45, synthetic black iron oxide, Nbutyl alcohol, SDA-3A alcohol, FD&C Blue No.2 Aluminum Lake, FD&C Red No.40 Aluminum Lake, FD&C Blue No.1 Aluminum Lake, and D&C Yellow No.10 Aluminum Lake.

Clinical Pharmacology General

In animal studies, Flutamide demonstrates potent antiandrogenic effects. It exerts its antiandrogenic action by inhibiting androgen uptake and/or by inhibiting nuclear binding of androgen in target tissues or both. Prostatic carcinoma is known to be androgen-sensitive and responds to treatment that counteracts the effect of androgen and/or removes the source of androgen, e.g., castration. Elevations of plasma testosterone and estradiol levels have been noted following Flutamide administration. **Pharmacokinetics**

Absorption

Analysis of plasma, urine, and feces following a single oral 200 mg dose of tritium-labeled Flutamide to human volunteers showed that the drug is rapidly and completely absorbed. Following a single 250 mg oral dose to normal adult volunteers, the biologically active alpha-hydroxylated metabolite reaches maximum plasma concentrations in about 2 hours, indicating that it is rapidly formed from Flutamide. Food has no effect on the bioavailability of Flutamide.

Distribution

In male rats administered an oral 5 mg/kg dose of14C-Flutamide neither Flutamide nor any of its metabolites is preferentially accumulated in any tissue except the prostate. Total drug levels were highest 6 hours after drug administration in all tissues. Levels declined at roughly similar rates to low levels at 18 hours. The major metabolite was present at higher concentrations than Flutamide in all tissues studied. Following a single 250 mg oral dose to normal adult volunteers, low plasma concentrations of Flutamide were detected. The plasma half-life for the alpha-hydroxylated metabolite of Flutamide is approximately 6 hours. Flutamide,*in vivo*, at steady-state plasma concentrations of 24 to 78 ng/mL, is 94% to 96% bound to plasma proteins. The active metabolite of Flutamide, in vivo, at steady-state plasma concentrations of 1556 to 2284 ng/mL, is 92% to 94% bound to plasma proteins. **Metabolism**

The composition of plasma radioactivity, following a single 200 mg oral dose of tritium-labeled Flutamide to normal adult volunteers, showed that Flutamide is rapidly and extensively metabolized, with Flutamide comprising only 2.5% of plasma radioactivity 1 hour after administration. At least six metabolites have been identified in plasma. The major plasma metabolite is a biologically active alphahydroxylated derivative which accounts for 23% of the plasma tritium 1 hour after drug administration.

The major urinary metabolite is 2-amino-5nitro-4-(trifluoromethyl)phenol.

Excretion

Flutamide and its metabolites are excreted mainly in the urine with only 4.2% of a single dose excreted in the feces over 72 hours.

Plasma Pharmacokinetics of Flutamide and HydroxyFlutamide in Geriatric Volunteers (mean ± SD)

	Single Dose Flutamide	HydroxyFlutamide	Steady-State Flutamide	HydroxyFlutamide
Cmax (ng/mL)	25.2 ± 34.2	894 ± 406	113 ± 213	1629 ± 586
Elimination half-life (hr)	---	8.1 ± 1.3	7.8	9.6 ± 2.5
Tmax (hr)	1.9 ± 0.7	2.7 ± 1.0	1.3 ± 0.7	1.9 ± 0.6
Cmin (ng/mL)	---	---	---	673 ± 316

Special Populations

Geriatric Following multiple oral dosing of 250 mg t.i.d. in normal geriatric volunteers, Flutamide and its active metabolite approached steady-state plasma levels (based on pharmacokinetic simulations) after the fourth Flutamide dose. The half-life of the active metabolite in geriatric volunteers after a single Flutamide dose is about 8 hours and at steady-state in 9.6 hours.

Race There are no known alterations in Flutamide absorption, distribution, metabolism, or excretion due to race.

Renal Impairment Following a single 250 mg dose of Flutamide administered to subjects with chronic renal insufficiency, there appeared to be no correlation between creatinine clearance and either Cmaxor AUC of Flutamide. Renal impairment did not have an effect on the Cmaxor AUC of the biologically active alpha- hydroxylated metabolite of Flutamide. In subjects with creatinine clearance of < 29 mL/min, the half-life of the active metabolite was slightly prolonged. Flutamide and its active metabolite were not well dialyzed. Dose adjustment in patients with chronic renal insufficiency is not warranted.

Hepatic Impairment No information on the pharmacokinetics of Flutamide in hepatic impairment is available (see **BOXED WARNING, Hepatic Injury**).

Women, Pediatrics Flutamide has not been studied in women or pediatric subjects. **Drug-Drug Interactions**

Interactions between Flutamide capsules and LHRH-agonists have not occurred. Increases in prothrombin time have been noted in patients receiving warfarin therapy (see **PRECAUTIONS**).

Clinical Studies

Flutamide has been demonstrated to interfere with testosterone at the cellular level. This can complement medical castration achieved with LHRH-agonists which suppresses testicular androgen production by inhibiting luteinizing hormone secretion.

The effects of combination therapy have been evaluated in two studies. One study evaluated the effects of Flutamide and an LHRH-agonist as neoadjuvant therapy to radiation in stage B2-C prostatic carcinoma and the other study evaluated Flutamide and an LHRH-agonist as the sole therapy in stage D2prostatic carcinoma.

Stage B2-C Prostatic Carcinoma

The effects of hormonal treatment combined with radiation was studied in 466 patients (231 Flutamide capsules + goserelin acetate implant + radiation, 235 radiation alone) with bulky primary tumors confined to the prostate (stage B2) or extending beyond the capsule (stage C), with or without pelvic node involvement. In this multicentered, controlled trial, administration of Flutamide capsules (250 mg t.i.d.) and goserelin acetate (3.6 mg depot) prior to and during radiation was associated with a significantly lower rate of local failure compared to radiation alone (16% vs. 33% at 4 years, $P < 0.001$). The combination therapy also resulted in a trend toward reduction in the incidence of distant metastases (27% vs. 36% at 4 years, $P = 0.058$). Median disease-free survival was significantly increased in patients who received complete hormonal therapy combined with radiation as compared to those patients who received radiation alone (4.4 vs 2.6 years, $P < 0.001$). Inclusion of normal PSA level as a criterion for disease-free survival also resulted in significantly increased median disease-free survival in patients receiving the combination therapy (2.7 vs. 1.5 years, $P < 0.001$).

Stage D2Prostatic Carcinoma

To study the effects of combination therapy in metastatic disease, 617 patients (311 leuprolide + Flutamide, 306 leuprolide + placebo) with previously untreated advanced prostatic carcinoma were enrolled in a large multicentered, controlled clinical trial.

Three and one-half years after the study was initiated, median survival had been reached. The median actuarial survival time was 34.9 months for patients treated with leuprolide and Flutamide versus 27.9 months for patients treated with leuprolide alone. This 7 month increment represents a 25% improvement in overall survival time with the Flutamide therapy. Analysis of progression-free survival showed a 2.6 month improvement in patients who received leuprolide plus Flutamide, a 19% increment over leuprolide and placebo.

Indications and Usage for Flutamide

Flutamide capsules are indicated for use in combination with LHRH-agonists for the management of locally confined Stage B2-C, and Stage D2 metastatic carcinoma of the prostate. **Contraindications**

Flutamide capsules are contraindicated in patients who are hypersensitive to Flutamide or any component of this preparation.

Flutamide capsules are contraindicated in patients with severe hepatic impairment (baseline hepatic enzymes should be evaluated prior to treatment).

10.0 Disposition of Data

Clinical records for all subjects studied including history and physical findings, laboratory and clinical data, and operative and dosimetric records are to be maintained by the investigators in a secure location at Johns Hopkins Cancer Center. Any records that are stored electronically will be password protected and only those who are involved in the research will have a password. These records are to be stored for a minimum of 5 years after last clinical visit.

11.0 Modification of the Protocol

All revisions or amendments (that are not required for immediate patient safety) to the protocol must be approved by the Johns Hopkins Institutional Review Board and by the Human Subjects Research Review Board (Department of Defense's IRB) prior to implementation.

12.0 Departure from the Protocol

If there is a departure from the Clinical Protocol, the Principal Investigator will notify in writing both the local IRB at Johns Hopkins and the HSRRB at the time of annual review (continuing review). The research coordinator will keep a log of all deviations/departures that occur on this project and this log will be reviewed by the research team on a monthly basis. During the review the research team will discuss corrective action plans to minimize future deviations/departures. If there are departures to the protocol that affect patient safety, the principal investigator will notify in writing the IRB within 24 hours of discovering the departure/deviation. Once a patient is 'off study', their treatment and followup plan will conform to what is determined to be in their best interest at that point, and no longer according to the protocol.

13.0 Criteria for Withdrawal from the Study

Patients may be withdrawn from the study for the following reasons: a)
Consent for participation is withdrawn

b) Noncompliance to study procedures

NOTE: Subjects will not be removed from the study should they require additional treatments due to worsening or recurrent cancer.

14.0 Roles and Responsibilities of Study Personnel

Principal Investigator: Oversees all aspects of the trial. Recruits and consents patients and administrates protocol specific procedures. Provides medical care to research subjects during the conduct of the study. Follows and advises regarding the treatment of adverse events. Reports SAE's to the JHM-IRB within the required time frame. Amends the trial as necessary to reflect unforeseen adverse events, new scientific data, and for the general integrity of the study. Monitors the trial and is ultimately responsible for the conduct of protocol.

Co-Investigators: If a physician**:** can recruit and consent patients and can administrate protocol specific procedures. Can provide medical care to research subjects during the conduct of the study. Has input on the course of action for adverse events.
If not a physician**:** Collaborates with the Principal Investigator according to area of expertise.

Research Nurses: Can consent patients. Executes protocol specific procedures requiring nursing qualifications. Provide nursing care to research subjects during the conduct of the study.

Data Manager/Study Coordinator: Collects data from subject's medical records and codes it onto the study's case report forms. Notifies principal investigator of any deviations that he/she finds while managing the data. Prepares annual IRB renewals and termination report upon study completion, assists with management of regulatory issues governing the trial. Monitors the trial.

15.0 Ethical and Regulatory Considerations

15.1 IRB: Prior to initiating the study, the Principal Investigator must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study protocol become necessary, protocol amendment will be submitted in writing to the IRB by the Principal Investigator for IRB approval prior to implementation.

15.2 Informed Consent: All potential candidates for the study will be given a copy to read of the Informed Consent for the study. The investigator will explain all aspects of the study in lay language and answer all the candidate’s questions regarding the study. If the candidate desires to participate in the study, he/she will be asked to sign the Informed Consent. No study procedures will be performed on a patient until after they have signed the informed consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

15.3 The principal investigator will ensure that the study is conducted in compliance with the

protocol and according ICH Guidelines for Good Clinical Practices, the Declaration of Helsinki, and all regulatory and institutional requirements, including those for patient privacy, informed consent, Institutional Review Board approval and record retention.

16.0 Study Compliance – Monitoring Plan

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring.

Interim analysis of toxicity, outcome and ongoing scientific investigations will be performed at least annually by the Sidney Kimmel Comprehensive Cancer Center Data Safety Monitoring Board (SKCCC DSMB). The SKCCC DSMB Recommendation letter will state the timeline for the next required review. The SKCCC DSMB will review aspects of this trial that are outlined in the responsibilities section of the Data and Safety Monitoring Board (DSMB) Guidance. If the committee decides that amendments should be made to this trial, recommendations will be made in writing to the Study Principal Investigator. The study team will submit modifications to the IRB within 60 days of receipt from the DSMB. The Associate Director of Clinical Research, will arbitrate any disagreements between the DSMB and the study Principal Investigator. These changes may include early termination of accrual if deemed appropriate.

17.0 References

1. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). *Prostate Cancer, Version 3.2016* (2016). Available at: https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf. (Accessed: 1st January 2016)
2. Fosså, S. D. *et al.* Ten- and 15-yr Prostate Cancer-specific Mortality in Patients with Nonmetastatic Locally Advanced or Aggressive Intermediate Prostate Cancer, Randomized to Lifelong Endocrine Treatment Alone or Combined with Radiotherapy: Final Results of The Scandinavian. *Eur. Urol.* (2016). doi:10.1016/j.eururo.2016.03.021
3. Coen, J. J., Zietman, A. L., Thakral, H. & Shipley, W. U. Radical radiation for localized prostate cancer: local persistence of disease results in a late wave of metastases. *J. Clin. Oncol.* **20,** 3199– 205 (2002).
4. Haffner, M. C. *et al.* Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat. Genet.* **42,** 668–675 (2010).
5. Haffner, M. C., De Marzo, A. M., Meeker, A. K., Nelson, W. G. & Yegnasubramanian, S. Transcription-induced DNA double strand breaks: both oncogenic force and potential therapeutic target? *Clin. Cancer Res.* **17,** 3858–64 (2011).
6. Hedayati, M. *et al.* Androgen deprivation followed by acute androgen stimulation selectively sensitizes AR-positive prostate cancer cells to ionizing radiation. *Clin. Cancer Res.* **22,** 3310–3319 (2016).
7. Niopas, I. & Daftsios, A. C. Determination of 2-hydroxyflutamide in human plasma by highperformance liquid chromatography and its application to pharmacokinetic studies. *J. Chromatogr. B Biomed. Sci. Appl.* **759,** 179–183 (2001).
8. Aryee, M. J. *et al.* DNA Methylation Alterations Exhibit Intraindividual Stability and Interindividual Heterogeneity in Prostate Cancer Metastases. *Sci. Transl. Med.* **5,** 169ra10169ra10 (2013).

9. Yegnasubramanian, S., Lin, X., Haffner, M. C., DeMarzo, A. M. & Nelson, W. G. Combination of methylated-DNA precipitation and methylation-sensitive restriction enzymes (COMPARE-MS) for the rapid, sensitive and quantitative detection of DNA methylation. *Nucleic Acids Res.* **34,** e19– e19 (2006).
10. Yegnasubramanian, S. *et al.* DNA Hypomethylation Arises Later in Prostate Cancer Progression than CpG Island Hypermethylation and Contributes to Metastatic Tumor Heterogeneity. *Cancer Res.* **68,** 8954–8967 (2008).
11. Yegnasubramanian, S. *et al.* Chromosome-wide mapping of DNA methylation patterns in normal and malignant prostate cells reveals pervasive methylation of gene-associated and conserved intergenic sequences. *BMC Genomics* **12,** 313 (2011).
12. Haffner, M. C. *et al.* Tracking the clonal origin of lethal prostate cancer. *J. Clin. Invest.* **123,** 4918– 22 (2013).
13. Weier, C. *et al.* Nucleotide resolution analysis of TMPRSS2 and ERG rearrangements in prostate cancer. *J. Pathol.* **230,** 174–83 (2013).