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Phase I feasibility trial of preoperative adjuvant stereotactic body radiotherapy for patients at high risk of local failure after prostatectomy

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Clinical Rationale: Prostate cancer patients with pathologic T3 disease, extensive positive margins, or high gleason grade are at elevated risk of local failure after prostatectomy (1–3). Adjuvant radiation improves clinically meaningful outcomes for patients at high risk of local failure after radical prostatectomy and is a standard of care (2). Adjuvant radiation, typically delivered postoperatively, is clinically underutilized (4). Preoperative adjuvant radiotherapy, if clinically feasible, would be a subject of interest for future clinical trials.

Preoperative adjuvant radiotherapy offers the following additional advantages: (1) reduced radiation dose to normal tissues; (2) lower prescription dose; (3) ability to utilize stereotactic body radiotherapy (SBRT); (4) patient convenience; (5) radiobiologic advantage of high dose per fraction treatment delivery. Furthermore, multiparametric (mp) MRI can predict extraprostatic extension (EPE) and seminal vesicle invasion (SVI) with 90% specificity (4), thereby offering a non-invasive method of selecting patients for preoperative adjuvant radiation. Prior clinical trials have evaluated the safety of, and identified a safe dose for, preoperative radiotherapy in prostate cancer. The feasibility of pre-operative adjuvant radiotherapy has yet to be evaluated.

Hypothesis: Preoperative adjuvant radiotherapy delivered by SBRT prior to prostatectomy in patients at high risk of local failure is feasible.

Design: This is a single arm Phase I feasibility study in prostate cancer patients at high risk for recurrence after prostatectomy.

- Patients will receive 3 fractions of 8Gy delivered by SBRT over 1-2 weeks, 2-4 weeks prior to prostatectomy and will come to clinic for standard follow- up for one year after surgery.

Primary Objective:

To assess feasibility of pre-operative SBRT in prostate cancer patients after prostatectomy. We consider the trial feasible when the estimated treatment completion rate for an enrolled patient is 75%. A patient is considered to have completed treatment if they received the prescribed radiation and undergo prostatectomy within 4 weeks of completing radiotherapy.

Secondary Objectives:

To assess acute toxicity (CTCAE v 4.0) and quality of life (EPIC and IPSS)

Correlative molecular analyses of tumor tissue and serum markers in patients that have undergone SBRT

Translational Significance: From laboratory studies we already know that the response of prostate cancer cells and tissues to high dose per fraction RT (8 Gy) differs from that to conventional dose per fraction RT (1.8-2 Gy) in ways beyond what can be expected from simple excess tumor cell death alone (6–8). We have evidence that the local and systemic immune responses that may be generated towards radiation- damaged prostate tumors after SBRT may be responsible for the discrepancy (8). However, the very conditions that promote tumor immunity may also enhance radiation- induced reprogramming of cancer towards a stem cell phenotype. How these differing outcomes play out in individual high-risk patients may be critical to the clinical outcome. The correlative analyses of this study will examine the tumor micro-environmental changes that might drive these divergent responses. This trial of prostate SBRT followed by prostatectomy offers a unique translational opportunity to study the radiobiology of SBRT in resected tissues.

Protocol Overview

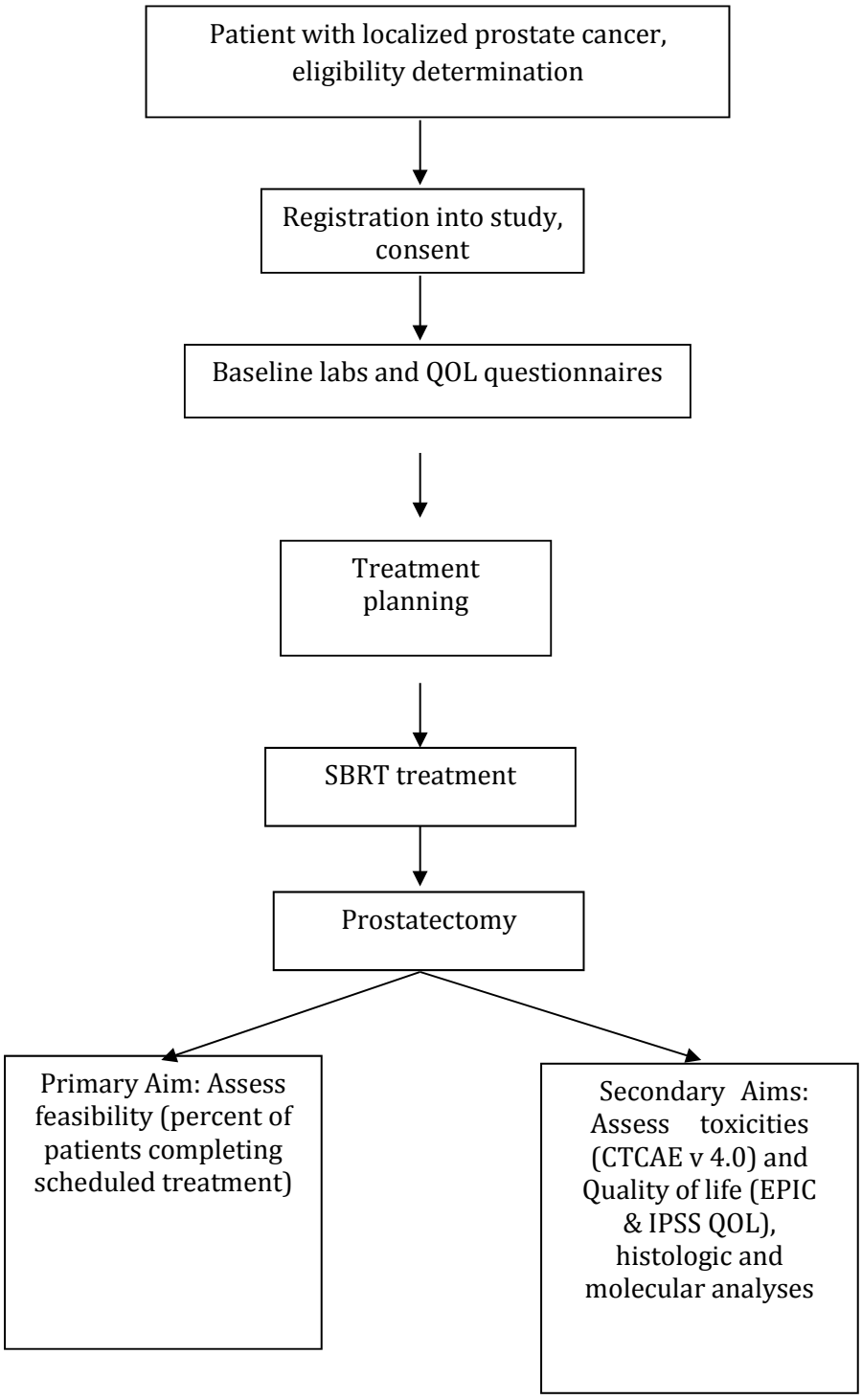
SBRT	Prostatectomy	Tests at each Follow-up
24Gy in 3 fractions (8 Gy per fraction)	Open or robotic assisted prostatectomy within 4 weeks (preferable 2 weeks) of SBRT	CTCAE toxicity scores, QOL-EPIC & IPSS scores, blood (including PSA)

Study Design: Prospective Phase I
Sample size: 12 patients, single-institutional
Outcome: Feasibility assessment (percent of patients completing scheduled treatment) Treatment: SBRT, prostatectomy
RT targets: Prostate PTV = prostate and SV (only if involved on MRI or biopsy)

Target Prescription Doses:
Prostate PTV 24 Gy in 3 fractions, 8 Gy per fraction

RT Technique: image-guided SBRT via robotic radiosurgery, IMRT, or VMAT, with daily and intra-fraction Image-guidance (MRI-based, fiducial-based or CBCT-based)

Trial Flowchart



Background

Prostate cancer is the most common non-cutaneous malignancy in men and second leading cause of cancer death in men (9). There are more than 230,000 incident cases per year and 30,000 deaths in the United States – the vast majority of which are from metastatic castrate resistant prostate cancer. However, more than 90% of men with metastatic prostate cancer are initially diagnosed with localized, not metastatic, prostate cancer. Therefore, improving cancer care for localized prostate cancer that is at high risk for recurrence is likely to reduce mortality.

The standard of care for localized prostate cancer is prostatectomy or definitive radiotherapy ± androgen deprivation therapy (ADT) (10). Patients who undergo surgery and have adverse features of positive margins, EPE, or SVI have up to 45-75% risk of biochemical failure within 5 years. Patients that undergo prostatectomy and have pathologic T stage 3 or greater or positive margins benefit from additional adjuvant therapy (2,3). Three randomized trials demonstrate improved progression free survival with the addition of adjuvant radiotherapy after prostatectomy in patients with pathologic T3 or positive margins: SWOG 8794, EORTC 22911, and ARO 96–02. SWOG 8794 was powered to detect a survival advantage and it did (2). As such, adjuvant radiotherapy with prostatectomy is a standard of care for patients with adverse features of pT3 (EPE or SVI),positive margins, or Gleason Grade 8-10 (2015 NCCN guidelines).

Prostate cancer imaging techniques have advanced such that prostate mp MRI can detect EPE and SVI with 90% specificity (5).

Only 15% of patients who meet the criteria for adjuvant radiation actually undergo this treatment (5). This low utilization may result from the inconvenience associated with seven to eight weeks of daily treatments, toxicity due to normal tissue irradiation, and the possibility that later salvage radiation at PSA recurrence might offer an alternative. The latter is unknown and is the subject of current investigations. The safety of pre- operative adjuvant radiotherapy has been evaluated in clinical trials and a safe biologically effective dose has been identified (12–14). Pre-operative adjuvant radiotherapy, if shown to be feasible, would be a subject of interest for a larger clinical trial.

Prior Studies of pre-operative radiotherapy in prostate cancer assessing safety

Duke University undertook a phase I dose escalation trial of pre-operative fractionated intensity modulated RT followed by prostatectomy (12). All patients were treated to the prostate and seminal vesicles to 39.6, 45, 50.4, and 54 Gy in fractions of 1.8 Gy. The pelvic nodes were also treated, but to a maximal dose of 45 Gy. No intraoperative morbidity was observed. Acute toxicity was limited to Grade 1 (GU and GI). Late toxicity, primarily GU, was within the range expected for adjuvant post-operative RT.

The conclusion was that 54 Gy is a safe dose for preoperative RT followed by prostatectomy.

Princess Margaret Hospital undertook a phase I trial of 15 patients for pre-operative RT followed by prostatectomy within four weeks of completing RT (15, 16). Conformal RT technique (one patient was treated with IMRT) was used to deliver 5 fractions of 5 Gy without image guidance. There were no intraoperative complications, no late GI toxicity. Two cases of long-term urinary incontinence were observed. Overall, GU toxicity was within the range expected for adjuvant post-operative RT.

Notably, our study would utilize modern image guidance and precise treatment volumes, which have been shown to reduce GU and GI toxicity in prostate radiotherapy as compared to conformal techniques.

Selection of RT dose for the current study

The preceding studies evaluating RT prior to prostatectomy demonstrate safety of pre-operative RT at doses of up to 54 Gy in 30 fractions (12). The critical organs at risk in prostate RT are the rectum and bladder. The biological effectiveness of RT for a given physical dose varies between tissues; different tissues (including tumor) are differentially responsive to dose per fraction (quantified by the alpha-beta ratio specific to the tissue or tumor) (15). In the rectum and bladder (alpha-beta ratio of 3-4) (16,17), an RT regimen of 24 Gy delivered in 3 fractions of 8 Gy is biologically equivalent to a dose less than 54 Gy delivered in 30 fractions of 1.8 Gy (the dose previously shown to be safe when delivered to the prostate and seminal vesicles prior to prostatectomy). However, because prostate tumors have a greater sensitivity to changes in dose per fraction than the rectum and bladder (alpha-beta ratio of 1.5) (18, 19), 24 Gy delivered in
3 fractions of 8 Gy deposits a higher biologically effective dose than 54 Gy in 30 fractions in prostate tumor tissue. A dose of 24 Gy delivered in 3 fractions is biologically equivalent in prostate tumor tissue to a standard post-operative adjuvant dose of 70.2 Gy delivered in fractions of 1.8 Gy.

In summary, our selected dose is: 1- based on prior studies identifying a safe dose; and, 2- equivalent to the standard adjuvant RT dose delivered postoperatively. Furthermore, delivering the radiation by SBRT using modern image guidance systems and precise treatment volumes is expected to further reduce toxicity as compared to the prior studies which did not use this delivery technique.

Biologic rationale for combination of preoperative adjuvant radiation delivered by SBRT with prostatectomy

The goal of adjuvant prostate radiotherapy is to eradicate residual disease associated with EPE and positive margins. The target is therefore the prostate surgical bed.

However, precise delineation of the clinical target volume (CTV) of the prostate bed is complicated by changes in anatomy due to surgery. Not surprisingly, controversy exists in the optimal target volume. The CTV must include significant portions of the posterior bladder wall and extend to the anterior rectal wall, exposing normal, healthy tissues to full dose radiation. A standard post-operative adjuvant dose is 70.2 Gy delivered in 39 fractions of 1.8 Gy.

Prostate cancer is highly sensitive to dose per fraction (the prostate cancer alpha-beta ratio of 1.5 is much smaller than 10, which is typical of other malignancies) (15). Consequently, there is a biologic rationale to use fewer, but relatively larger sized fractions (~8 Gy per fraction) of radiation in prostate cancer, compared to conventional fractions of 1.8 to 2 Gy that are used for most cancers (20).

Definitive radiotherapy for prostate cancer has benefited greatly from technical improvements in image guidance and treatment delivery, enabling large fraction sizes to be safely delivered by stereotactic body radiotherapy (SBRT = delivery of up to five fractions with tight margins under image guidance). The CTV for prostate SBRT is the prostate alone (and seminal vesicles if involved) and the planning target volume (PTV) is a small expansion. Unfortunately, the use of SBRT in the postoperative setting (adjuvant or salvage) is frustrated by the lack of consensus of the ideal CTV and the necessity of including significant volumes of normal tissues.

Therefore, there is a two-fold rationale to utilize preoperative, adjuvant SBRT in patients at risk for local failure after prostatectomy: 1- pre-operative RT delivered by SBRT is more convenient for patients (only 3 treatments), which would likely increase utilization of adjuvant RT if shown to be clinically feasible; 2- delivery of large fraction sizes are radio-biologically advantageous.

Clinical - Translational Relevance

Empiric clinical data has demonstrated that prostate adenocarcinoma is exquisitely sensitive to changes in fractional size of radiation dose to a degree larger than even surrounding normal tissues (19, 19). It follows that there is no benefit derived from fractionating radiation dose to the conventional daily dose of 2 Gy during treatment, and that use of larger fractions will increase the therapeutic benefit with more tumor control at the same or decreased toxicity. This is in contrast to most other cancer types where conventional fractionation to 1.8-2 Gy per day is justified because surrounding normal tissues, but not tumors, are spared.

SBRT is the technique of choice to deliver such higher doses. Surprisingly, though, our understanding of the biology behind SBRT have not kept up with the clinical success of this modality due to a dearth of translational studies using specimens from patients treated with SBRT. This Phase I trial of preoperative SBRT in patients at high risk of local recurrence following prostatectomy offers the possibility of studying the radiobiology of SBRT in the clinic. It also lends itself to examination of the role of radiation-

induced immunity in preventing recurrences in high-risk disease and to the role of EMT in promoting recurrences. The dynamic nature of the relationships between these phenomena is of particular interest to our team.

In vitro and in vivo work has suggested that high dose per fraction RT alters the extent and the type of damage to DNA and to vascular structures including endothelial cells, and it seems to generate stronger anti-tumor immunity. We already know that irradiation generates an immunogenic cancer cell death, in part by inducing genetic programs that signal “danger” to the immune system (6). SBRT may therefore drive immune recognition and tumor immunity of potentially systemic proportions (8). It starts with the generation of a pro-inflammatory microenvironment with up-regulation of MHC and co- stimulatory molecules that can enhance tumor antigen cross-presentation by dendritic cells and that broadens the antigenic repertoire. Progenitor cells of mesenchymal, endothelial, myeloid and lymphoid origin get mobilized to infiltrate the irradiated area. What is important here is that according to preclinical evidence, doses considerably higher than the conventional 1.8-2 Gy are required to generate high enough levels of pro-inflammatory cytokines and superior anti-tumor responses. Based on our experience with clinical and pre-clinical tumor models, we hypothesize that pre- operative, prostate SBRT with 3 fractions of 8 Gy given over a relatively short time will lead to measurable immunological changes, locally and systemically. Understanding these changes could inform the use of immunomodulatory therapies in locally-advanced as well as metastatic prostate cancer. Unfortunately, the same pro-inflammatory conditions may drive reprogramming towards a more resistant cancer stem cell phenotype that could increase the likelihood of recurrence in high-risk patients. The balance between these forces and their correlation, if any, in individual high-risk patients will be investigated.

The translational component of this trial seeks: 1. To investigate the radiobiology of SBRT in prostate cancer using resected prostate tumor tissue, and 2. To evaluate the nature of the immune response to prostate cancer generated by SBRT. These aims are included within the secondary objectives of this trial.

Scientific impact

RT causes genotoxicity in irradiated tissue, which is a primary cause of cancer cell killing (21). More recent evidence suggests that additional radiation-induced pathways are at play which drive immune recognition and tumor-immune eradiation with potentially systemic consequences, especially after high dose per fraction RT (6–8). The promising synergy between RT and the immune system is underscored by a multitude of ongoing clinical trials that combine local irradiation with systemic immunomodulatory therapeutics. Translational studies correlating such systemic and intratumoral responses using freshly irradiated tissue, however, are extremely rare. Laboratory studies suggests that the doses used in prostate SBRT are close to the optimal fraction sizes to generate such measurable immunological changes. Therefore, this pre-operative prostate SBRT trial offers a unique opportunity to study the radiobiology of SBRT in the clinic, one that simply cannot be ignored.

Another level of complexity arises from the fact that RT may induce markers of EMT and neuroendocrine differentiation in prostate and other cancers, potentially promoting treatment resistance (22,23). If prostate radiotherapy drives such de novo transformations above what exists anyway, then strategies targeting these pathways would be particularly effective when combined with radiation. What's more, the hypothesis is that EMT transformations are promoted by pro-inflammatory conditions, which would suggest an alternative, and less favorable outcome for high-risk patients after receiving SBRT. Resolving the relative importance of EMT viz-a-viz immune system activation in individual patients is a novel and important long-term goal of this study. It will generate insight by studying both EMT and pro-inflammatory and immune SBRT-induced changes within the radiation-damaged microenvironment in vivo using IHC and transcriptomics. Currently, these changes are largely unknown. Therefore, we believe the correlative genomic analyses we have proposed would, at minimum, be hypothesis generating for future investigations with high translational impact.

Study design:

This is a single arm, Phase I feasibility study of SBRT followed by prostatectomy within 4 weeks for patients with prostate cancer at high risk of local failure after prostatectomy. A patient is considered to have completed treatment if they received the prescribed radiation and undergo prostatectomy within 4 weeks of completing radiotherapy.

NUMBER OF PATIENTS;

12 patients in 3 years with recruitment at UCLA Jonsson Comprehensive Cancer Center

Objectives:

Primary Objective: To assess feasibility of pre-operative SBRT in prostate cancer patients at high risk for recurrence after prostatectomy. We consider the trial feasible when the estimated treatment completion rate for an enrolled patient is 75%. A patient is considered to have completed treatment if they received the prescribed radiation and undergo prostatectomy within 4 weeks of completing radiotherapy.

- **Secondary Objectives**:
    - To assess safety and acute toxicity of SBRT followed by prostatectomy. This will be based on CTCAE version 4.0 and patient reported quality of life (EPIC and IPSS questionnaires).
    - Investigation of the radiobiology of SBRT in prostate cancer using resected prostate tumor tissue. For the correlative genomic analyses, prostate lesions at the time of prostatectomy will be processed for RNA and exome sequencing and compared to existing sequencing data for localized prostate cancers. We will utilize both supervised and unsupervised analyses of

Pathway activation from RNA-seq data sets in order to determine which pathways are enriched after irradiation.

- Evaluation of the nature of the immune response to prostate cancer generated by SBRT.
  - Systemic immune response. Blood that is collected pre SBRT, between 0 and 4 weeks after SBRT, and at 3 and 6 month intervals after SBRT will be assessed as follows: PBMCs will be isolated over Ficoll-gradients and stored frozen until analysis. Lymphoid and myeloid subset phenotyping will be assessed by antibody staining and multi- color flow cytometry (LSR-Fortessa, BD). Circulating cytokines, PGE2, prostate-tumor specific antibodies, HMGB-1 protein and the tryptophan/kynurenine ratio in plasma will be quantified by ELISA. Tumor-specific CD4 and CD8 T cell activities to known HLA-matched, immunodominant, tumor-associated antigens will be assessed ex vivo by IFN γ -ELISPOT and dextramer staining. The tumor antigens of choice will include PSA and PSMA.
  - Local immune response. The immune response at the tumor site will be visualized through immunohistochemistry (IHC), with particular focus on T cells (CD3+), T regulatory cells (CD4+ CD25hi), tumor infiltrating myeloid-derived suppressor cells (CD11b+ Gr-1+ CD15+) and tumor-associated macrophages (CD11b+F4/80+). Levels of CSF1/CSF1R, tumor MHC I, PD-1 and PD-L1 are also of interest as are markers for EMT (vimentin, N-cadherin, E-canderin), for neuroendocrine differentiation (chromogrannin A, neuron specific enolase), AR protein and p53. Comparisons will be made to pre- treatment biopsy material when possible and to tissue microarrays of non-irradiated prostatectomy specimens.

Eligibility Criteria

- Histologically confirmed primary non-metastatic adenocarcinoma of the prostate
- Patient desires and is medically fit to undergo prostatectomy
- Age ≥ 18
- KPS ≥ 70
- Patients on ADT are allowed
- For confirmation of high risk local failure status, patients will have any one of the following:
  - CT or MRI demonstrating SVI or EPE within 1 year of enrollment into the study
  - Pre-biopsy PSA ≥ 20
  - Gleason score 7-10 (Gleason 7 must be 4+3), presence of any Gleason 5 (even if a tertiary score) as determined at diagnostic biopsy

  - Gleason score 7 and >50% of biopsy cores positive for prostate cancer
  - Clinical stage ≥ T3 (staging by imaging acceptable)
- An image-guided biopsy (via Artemis Ultrasound with MRI co-registration) is encouraged but not required if not performed as standard of care biopsy

Ineligibility Criteria

- Distant metastases, based upon:
  - CT scan or MRI of the abdomen/pelvis or PSMA PET/CT within 120 days prior to registration and
  - Bone scan or PSMA PET/CT within 120 days prior to registration; if the bone scan is suspicious, a plain x-ray and/or MRI must be obtained to rule out metastasis prior to registration.

- Patient is unable or unwilling to sign consent.
- Patient is considered low-risk and would not have received adjuvant RT outside of this study

REGISTRATION PROCEDURES

General guidelines

Subjects will be identified by physicians in Urology or Radiation Oncology. The Study Coordinator will enter eligible patients on study centrally at the UCLA Jonsson Comprehensive Cancer Center. Subjects will be recruited through self- referral and the advice of their attending physician.

Registration process

A member of the research team will enroll the patient onto the trial. Consent will be obtained after a clear and thorough discussion between the patient and the principal investigator or any of the co-investigators in clinic. Any patients that are deemed by the principal investigator or co-investigators to be mentally or physically incapable of consent will not be included in the study.

Once a candidate has provided full consent, the following will be completed/collected to fully determine eligibility:

- Signed patient informed consent form
- HIPAA authorization form
- Medical history, clinical examination and consultations with Radiation

Oncology and Interventional Radiology departments

- Verification of clinical stage ≥ T3 (staging by imaging acceptable)
- Verification of no distant metastases, based upon:
    - CT scan or MRI of the abdomen/pelvis or PSMA PET/CT within 120 days prior to registration and
    - Bone scan or PSMA PET/CT within 120 days prior to registration; if the bone scan is suspicious, a plain x-ray and/or MRI must be obtained to rule out metastasis

REGISTRATION

Study team will register subject with the UCLA Data Safety Monitoring Board to assure eligibility, prior to undergoing any further study-related procedures.

PRIOR TO RADIATION TREATMENT

Prior to radiation treatment, approximately 60 mLs of blood will be drawn for serum translational biomarker study.

TREATMENT PLAN

Radiation Simulation:
Enrolled patients will undergo radiation simulation and planning as per routine for prostate cancer patients. A custom vacloc bag, alpha cradle, or equivalent immobilization device. A simulation scan will be performed. A pelvic mp MRI will be obtained if not available within the prior 4 months. The MRI will be utilized as a supplement for anatomical contour delineation. The urethra will be delineated with the MRI. These procedures are considered standard-of-care for prostate radiotherapy planning.

Radiation Planning (Target Areas):

- The study investigator will delineate the prostate, seminal vesicles on the pelvic simulation CT.
- The prostate PTV will consist of an expansion of the prostate gland up to 7mm in all directions, (expansion may be reduced to 3mm posteriorly).
- The SV PTV will consist of the contoured SV without expansion.
- For all PTVs, the Prescription Dose will be defined to cover 95% of the PTVs.
- Target Prescription Doses:
  Prostate 24 Gy in 3 fractions, 8 Gy per fraction
  SV (if involved) 24 Gy in 3 fractions, 8 Gy per fraction
- Technique: image-guided SBRT via robotic radiosurgery, IMRT, or VMAT, with daily and intra-fraction Image-guidance fiducial-based, CBCT-based, MRI-based.

Immediately after the 3rd (final) fraction of radiation treatment up to 4 weeks post SBRT, approximately 60 mls of blood will be drawn for serum translational biomarker study.

Prostatectomy:

Radical prostatectomy will be performed, either open or robot assisted, within 4 weeks after SBRT (preferably 2 weeks). After resection, the prostate is sectioned from base to apex into 5-7 slices depending on the size of the prostate. Slices 2 and 4 are kept fresh on ice, while all other slices are fixed in formalin and embedded in paraffin. A slide is cut and stained from the paraffin sections for pathologic diagnosis and the remaining tissue will be kept indefinitely in pathology and can be used for research.

Frozen section is prepared from the fresh tissue (slices 2 and 4) for rapid diagnosis. Part

of the fresh tissue is used for research and the remaining fresh tissue is frozen indefinitely and can be used for research.

Follow-up:

- 0 to 4 weeks after the final fraction of radiation, patients will have approximately 60 mLs of blood drawn for serum translational biomarker research. Patients will also be evaluated by their physician and Adverse Events and Concomitant Medications will be assessed. EPIC and IPSS questionnaires will also be administered at this time.
- Patients are followed every three months (± 4 weeks) for one year post surgery. At each follow-up visit, patients are evaluated for CTCAE (version 4.0) toxicity scores, and the EPIC and IPSS questionnaires will be administered. Blood draws (~5 mLs) to assess PSA are drawn at each of the follow-up visits unless PSA lab evaluations have already been done within 4 weeks of the study visit. In addition, at the 3 month and 6 month follow-up visits, approximately 60 mLs of blood will be drawn for serum translational biomarker study. After one year of follow-up, patients will be seen as per routine care.

OUTCOME MEASURES

Primary objective, feasibility: A patient is considered to have completed treatment if they received the prescribed radiation dose of 24 Gy delivered in 3 fractions of 8 Gy within a span of 2 weeks and subsequently undergo prostatectomy within 4 weeks of completing radiotherapy.

Secondary objective, safety and acute toxicity: Patients will be evaluated during RT, prostatectomy, and at intervals of 3 months following completion of treatment for one year. At each visit, patients will complete the EPIC and IPSS questionnaires and will be assessed for CTCAE v4 toxicities.

Secondary objective, correlative studies: Assessments of the systemic immune response will utilize blood that is collected pretreatment, after completion of SBRT, and at 3 and 6 months post treatment completion. The local immune response (intratumoral) will utilize the resected prostatectomy specimen, pre-treatment biopsies, and archived tissue arrays of prostatectomy specimens that did not undergo RT. RNA- and exome-sequencing of irradiated prostate tumors will utilize the resected prostatectomy specimens, which will be compared to existing sequencing analyses of localized prostate cancers (TCGA) matched for histology and Gleason grade.

STATISTICAL CONSIDERATIONS

Feasibility and sample size determination. Our primary objective is to determine feasibility of preoperative adjuvant stereotactic body radiotherapy for patients who are at high risk of local failure after prostatectomy. A confidence interval approach is used to estimate the sample size appropriate for this feasibility study (24, 25). We consider preoperative adjuvant stereotactic body radiotherapy feasible when the estimated treatment completion rate for an enrolled patient is at least 75%. For a two-sided 90% exact binomial confidence interval, a sample size of 12 patients yields a lower limit of the 90% confidence interval of at least 75% if the true proportion of patients completing treatment is 93%. A feasibility assessment of preoperative adjuvant stereotactic body radiotherapy is a required step prior to designing a larger trial.

Estimation of eligible patients and enrollment. Numbers of eligible patients are estimated based on projected patient populations. Eligible patients for the study are drawn from prostate cancer patients referred for treatment evaluation at UCLA Medical Center by the study investigators. This is anticipated to be 200 patients in 2016. At least 25% are anticipated to be eligible based on the risk classification of patients for our protocol (patients at high risk of recurrence). We estimate 75% of these patients will be surgical candidates. Standard treatments for these patients include either surgery (that includes adjuvant radiotherapy with or without androgen deprivation therapy) or definitive radiotherapy with concurrent androgen deprivation therapy. We estimate 40% of the patients will elect definitive radiotherapy with androgen deprivation. Of the remaining 60% of eligible patients, we anticipate that between 20 and 50% would be amenable to preoperative adjuvant radiotherapy. Our study is planned to accrue patients within three years. Therefore, there are sufficient numbers of patients eligible to participate in this study.

Measurements of serological markers (secondary objective). Circulating cytokines, PGE2, prostate-tumor specific antibodies, HMGB-1 protein and the tryptophan/kynurenine ratio in plasma will be quantified by ELISA. Tumor-specific CD4 and CD8 T cell activities to known HLA-matched, immunodominant, tumor-associated antigens will be assessed ex vivo by IFNγ-ELISPOT and dextramer staining. The tumor antigens of choice will include PSA and PSMA. A repeated measures analysis of variance will be used to assess differences in these serological markers over time. In addition to the overall time effect in the ANOVA model, we plan to test specific contrasts

between serological markers pre-treatment (baseline) and the first measurement post SBRT (pre surgery), where we anticipate to observe an increase in circulating tumor-specific CD4 and CD8 T cell activity.

Multivariate profile analyses may be used when patterns for various markers emerge. These statistical analyses will be performed using IBM SPSS Statistics for Windows, Version 20.0. (Armonk, NY) statistical software.

Measurements of intra-tumoral and intra-prostatic markers (secondary objective). The immune response at the tumor site will be visualized through immunohistochemistry (IHC), with particular focus on T cells (CD3+), T regulatory cells (CD4+ CD25hi), tumor infiltrating myeloid-derived suppressor cells (CD11b+ Gr-1+ CD15+) and tumor-associated macrophages (CD11b+F4/80+). Levels of CSF1/CSF1R, tumor MHC I, PD-1 and PD-L1 are also of interest as are markers for EMT (vimentin, N- cadherin, E-canderin), for neuroendocrine differentiation (chromogrannin A, neuron specific enolase), AR protein and p53. IntMax (scale of 0-300) = (% of positive tumor cells [scale of 0-100]) X (intensity of staining based on 0-3 scale [scale of 0-3]) will be assessed for each marker. Comparisons in markers will be made between: 1- post SBRT prostatectomy specimens vs biopsy material pre-SBRT; and 2- post SBRT prostatectomy specimens vs archival tissue of prostatectomy specimens (that did not undergo RT).

For comparison between post SBRT prostatectomy specimens and biopsy material pre-SBRT, average IntMax will be compared using a paired t-test or Wilcoxon signed-rank test (if the IntMax scores do not follow a normal distribution). For comparison between post SBRT prostatectomy specimens and archived material, average IntMax will be compared using a t-test or Wilcoxon rank-sum test (if the IntMax scores do not follow a normal distribution).

RNA and DNA exome sequencing (secondary objective). Quality control will be performed on the raw sequence data from RNA-seq experiments, which contain multiple short-read sequences with Phred quality score information using FastQC and FASTX-Toolkit. Bowtie2 will be used to align reads, which will then be mapped to the reference genome by TopHat2. Raw reads from exome sequencing will be aligned via BWA and converted to BAM format, and processed with the Genome Analysis Toolkit software package. Comparisons will be made to existing sequencing data (TCGA) for localized prostate tumors matched for histology and Gleason grade. Functional annotation will be performed using Gene Ontology, KEGG, and Reactome.

Stopping Rules for Toxicity. Prior studies evaluating RT prior to prostatectomy demonstrate safety of pre-operative RT at doses of up to 54 Gy in 30 fractions (12). It is anticipated that toxicity in this study will be low because our dose is selected based on the prior studies.

Nevertheless, our trial will be stopped for toxicity if any treatment related toxicity of CTCAE v4.0 grade 3 or above is observed in more than two subjects during the study

period in the following categories: gastrointestinal, urinary, with the exception of temporary use of a catheter and urinary incontinence, which can occur after prostatectomy in the absence of radiotherapy.

DATA and SAFETY MONITORING:

The JCCC DSMB meets monthly to review all SAE reports for trials overseen by the JCCC DSMB. All SAE reports, which have been filed since the previous meeting, are presented to the committee for review.

For trials overseen by the JCCC DSMB, the DSMB reviews all dose-limiting toxicities (DLTs) for dose-escalation studies. Protocol suspensions and re-opening of accrual to the next cohort based on DLT evaluation fall under the purview of the DSMB.

For all JCCC oncology trials and TRIO-US studies where the JCCC DSMB has primary oversight, all SAEs shall be reported to the JCCC DSMB in a timely manner [ten days, two days for a death] regardless of relationship and expectedness. The JCCC ORC will review all submissions and the ORC staff will enter the information into the JCCC Clinical Trials database. Reports are generated for full JCCC DSMB review. For trials where the JCCC DSMB has primary DSMB review responsibility, the DSMB requires that the PI generate cumulative adverse event reports for quarterly, biannual or annual review.

The DSMB reviews each SAE report and determines whether or not protocol modifications are warranted to ensure subject safety. In this review, prior occurrences of similar toxicity with the therapy under study are taken into consideration, as well as the severity of the event and the likelihood that it was related to a study drug. The DSMB may recommend no changes to the study if the event is expected or related to other causes such as the subject's underlying condition. The DSMB may request an expert's advice of another non-Principal Investigator with national experience to support their deliberations and decisions.

The JCCC DSMB has the authority to recommend to the UCLA IRB the immediate halt to a study (i.e., discontinuation of any further treatment of enrolled subjects and discontinuation of enrollment of new subjects) should there be any serious unexpected toxicity that warrants further investigation.

Requests for single subject exceptions/waivers from the approved study protocol, including out of window procedures and eligibility deviations, must be reviewed and approved by a member of the DSMB. Each trial is assigned a primary and secondary reviewer who is responsible for reviewing each exception/waiver request for that trial. Approvals and disapprovals of the request are sent to the Principal Investigator via email and copied to the UCLA IRB. Requests for single subject exceptions/waivers are made by the Principal Investigator via email utilizing the "Single Subject Exception

Request Form."

JCCC DSMB correspondences are addressed to the Principal Investigator and copied to the UCLA IRB. Minutes of the DSMB meetings are maintained in a computer file.

Confidentiality: Each member of the JCCC DSMB is responsible for maintaining strict confidentiality of the study data. Members will not share any study data or information about the study with any individual external to the JCCC DSMB or the statistical working group for the study. The DSMB members may contact the statistical working group directly with questions regarding the operational details associated with the data analysis and summary presentations. Communication of deliberations or recommendations of the JCCC DSMB, either written or oral, should not be made outside of the Committee or the statistical working group. Outcome results are strictly confidential and must not be divulged to any non-member of the JCCC DSMB except in those cases where DSMB is required to inform the UCLA IRB of its determinations. Disclosure of outcome results to the IRB must only occur with written approval of the DSMB. A member who believes he or she may have a potential intellectual or financial conflict of interest during the course of review of the data must inform the chairperson of the DSMB. In such case, the meeting minutes will record the disclosure of the potential conflict of interest and that the individual recuse himself from the discussions and abstains from voting on the DSMB decision.

Level of Risk of a Study

All interventional clinical trials undergo scientific review by the Internal Scientific Peer Review Committee (ISPRC) which requires that a Data and Safety Monitoring Plan is in place before a trial can be approved to begin. For trials overseen by the JCCC DSMB, the JCCC DSMB will determine the degree of risk of the study and will ensure that there are procedures in place to ensure the safety of the subjects that are enrolled in the trial. The intensity level of study oversight is determined by the risk category. Some of the factors that are considered when assigning the Level of Risk category include:

- A biostatistical design and appropriate procedures for proper data management so that the information collected can be properly validated.
- Appropriate Serious Adverse Event reporting procedures must be in place.
- The study duration must be appropriate and must be based on a realistic rate of enrollment.
- Data collection and data management must be adequate to verify and ensure subject eligibility.

Assignment of risk

Assigning risk ensures that the data and safety monitoring is based on the level of risk (low, medium, or high) to ensure that the data and safety monitoring activities are appropriate. Below are some of the criteria used to make a decision regarding the assignment of risk:

- Expected duration of the study based upon the estimated rate of enrollment.
- Type of study population (e.g., children, geriatric)
- The procedures used in the trial are commensurate with the degree of risk.
- Adequate data management systems in place and appropriate case report forms
- Proper serious adverse event reporting procedures in place
- Proper biostatistical design and data analysis procedures in place.

Level of Risk

Level 2

Example of type of trial:

Institutional study for which IND is exempt by FDA or has an IND, but the drug is approved by the FDA for a different indication and is in Phase II or III. Examples are studies using commercially available agents for an unapproved indication based on standard regimen.

- Compliance Officer meets with PI/Staff prior to study initiation; review regulatory requirements and operating system. Compliance Officer provides real time monitoring to determine eligibility prior to enrollment onto the protocol.
- Real time QA monitoring of the subjects and data collection occurs for all subjects entered onto the trial.
- Comprehensive QA auditing within first year or first 10 subjects enrolled, whichever comes first. Subsequent audit frequency will be annually.
- Frequency of DSMB Summary Report is typically on a biannual basis or approximately every six months.

TABLE OF STUDY ASSESSMENTS

Assessment	Pre-Study	Pre-SBRT	SBRT	0-4 weeks post SBRT	Within 4 weeks of final SBRT	3 Month Follow-up (± 4wks)	6 Month Follow-up (± 4wks)	9 Month Follow-up (± 4wks)	12 Month Follow-up (± 4wks)
Informed Consent	X								
Demographics	X								
Medical History	X								
Initial RadOnc Consultation	X								
Confirmation of High Risk local failure status	X								
Surgical Evaluation & Clearance	X								
PSA	X (Pre-diagnostic biopsy)					X	X	X	X
Translational Research Blood Draw[1]		X		X		X	X		
Radiation Treatment			3 fxs within 1-2						
Prostatectomy					X				
EPIC Qxr	X (Anytime pre-tx)			X		X	X	X	X
IPSS Qxr	X (Anytime pre-tx)			X		X	X	X	X
Adverse Event Evaluation	X			X		X	X	X	X
Concomitant Medications	X			X		X	X	X	X
CT or MRI of Abdomen/Pelvis (within 120 days prior to registration) [3]	X[2]								
Bone scan (within 120 days prior to registration)	X[2]								
Treatment Planning scan		X							
Follow-up visit						X	X	X	X

[1] *Translational Research blood draw will consist of 5 x 10 ml EDTA lavender top tubes, 1 x 5 ml gold top tube and 1 x 5 ml CTAD blue top tube (a total of approximately 60 mLs)*

[2] *To verify no distant metastases, the subject must have CT or MRI of the abdomen/pelvis and a bone scan within 120 days prior to registration. If the bone scan is suspicious, a plain x-ray or MRI must be obtained to rule out metastasis.*

[3] *CT/MRI scan of Abdomen/Pelvis is not required if patient gets PSMA PET scan*

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