Phase II Trial of Neutron Radiotherapy with Concurrent Checkpoint Inhibitor Immunotherapy (pembrolizumab) in Patients with Advanced Urothelial Carcinoma

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Version: 01/08/2019

Study Summary

Title	*Phase II Trial of Neutron Radiotherapy with Concurrent Checkpoint Inhibitor Immunotherapy pembrolizumab) in Patients with Advanced Urothelial Carcinoma*
Short Title	*Neutron + Pembrolizumab in Advanced Urothelial Cancers*
Protocol Number	*CC 9940*
Phase	*II*
Methodology	*1 Arm Open-Label*
Study Duration	*2 yrs*
Study Center(s)	*University of Washington Medical Center*
Objectives	*To primary objective is to assess whether neutron radiation (with high relative biological effectiveness relative to standard photon radiation) to a single focus of disease in patients with advanced urothelial carcinoma, can improve the overall response rate to standard of care checkpoint inhibitor immunotherapy (pembrolizumab). The primary endpoint is the overall response rate per iRECIST. Secondary objectives are to evaluate toxicity, immune-related clinical responses and immune pharmacodynamic changes, progression-free survival, and overall survival.*
Number of Subjects	*20 evaluable subjects*
Diagnosis and Main Inclusion Criteria	*Patients diagnosed with advanced urothelial carcinoma, about to start checkpoint inhibitor immunotherapy (pembrolizumab) per standard of care, and eligible to undergo radiation to at least one site of disease with at least one additional site of disease that is measurable per RECIST criteria. Patient may receive radiation to asymptomatic sites of disease.*
Study Product, Dose, Route, Regimen	*All treatments are approved standard of care for patients with advanced urothelial carcinoma, including pembrolizumab and neutron radiation to a metastatic site. Dosing of pembrolizumab will be in accordance with their FDA label. Neutron radiation will be administered at a palliative dose/fractionation regimen to a site of disease that is either symptomatic, likely to become symptomatic, or likely to cause minimal toxicity with radiation treatment.*
Duration of administration	*Checkpoint inhibitor immunotherapy will be given per standard clinical care until patients develop progression or intolerable toxicity. Neutron radiation treatments will be given standard of care as well, for a maximum of 5 daily radiation treatments.*
Statistical Methodology	*Overall response rate will calculated as the percentage of patients achieving a PR or CR, and will be presented along with the 95% CI. Historical data suggest a response rate of 20%, and we hypothesize a true response rate of 40% with the proposed treatment. With 20 patients, we'll have 80% power to observe an estimated response rate that is statistically significantly higher (at the one-sided significance level of .05) than the fixed rate of 20%.*

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1 Introduction

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

Bladder cancer has seen success with immunotherapy, although life expectancy with metastatic bladder cancer remains poor:

This trial tests whether the addition of focal radiation can act as a "vaccine" by releasing tumor neoantigens, with the goal of augmenting the immune response to immunotherapy. Bladder cancer will be diagnosed in around 79,000 patients in the US in 2017 with around 17,000 deaths[1]. For patients with metastatic urothelial bladder cancer, FDA has approved five immune checkpoint inhibitors as of 8/12/2017 targeting the PD-1/PD-L1 pathway, including atezolizumab (PD-L1 inhibitor), nivolumab (PD-1 inhibitor), pembrolizumab (PD-1 inhibitor), avelumab (PD-L1 inhibitor), and durvalumab (PD-L1 inhibitor). However, outcome for these patients remain poor. For metastatic urothelial bladder cancer, median overall survival (OS) is around 12 months[2]. For patients who relapse after chemotherapy, median survival is worse, with few effective systemic options. A phase 2 trial with atezolizumab in patients with metastatic urothelial carcinoma that progressed after platinum-based chemotherapy showed an improvement in response from 10% historically to 15% with atezolizumab (p=.0058)[3]. Similarly, nivolumab was tested in a phase 2 trial of advanced urothelial carcinoma and showed 19.6% response with median OS 8.7 months[4]. Pembrolizumab was given to patients who progressed after platinum-based chemotherapy in a phase 3 trial versus standard second line chemotherapy, and showed an improvement in OS (10.3 months vs. 7.4 months, p=0.002), with objective response of 21.1% in the pembrolizumab group versus 11.4% in the chemotherapy group[5]. Pembrolizumab has also been tested in a phase 2 trial in the first line setting in patients who are ineligible for platinum-based chemotherapy, with an objective response rate of 24%[6]. Although these results are improvements over historical controls, response rates of 20% and median survivals of less than a year leave room for improvement.

Standard photon radiation can be combined with immunotherapy to augment anti-tumor activity in a variety of solid tumors:

Radiation causes release of tumor antigens and cytokines into the tumor microenvironment, which leads to an inflammatory response and infiltration of immune cells including T-cells (both cytotoxic and regulatory), dendritic cells, macrophages, and myeloid-derived suppressor cells (MDSCs)[7]. Peripheral blood from patients with metastatic melanoma receiving standard photon radiation to a single site of disease while on ipilimumab show that radiation induces an increase in percentage of CD4+ T-cells, ratio of CD8+ T-cells to Tregs, decrease in MDSCs, and increase in HLA-DR expression on monocytes[8,9]. Clinical reports have been published with standard photon radiation to one tumor causing an abscopal effect that leads to systemic regression of disease outside the radiation field, even in patients who have previously progressed on immunotherapy[8,10,11]. Although the exact mechanism of the radiation induced abscopal effect is unclear, published data supports it is partially due to T-cell effector function in the irradiated tumor, especially CD8+ T-cells[9,12]. There is also growing evidence that cytokine release is a major mechanism, which can lead to a decrease in MDSCs[8,13,14].

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

All of the published data on radiation and its ability to augment response to immunotherapy have been with standard photon radiation, which is sparsely ionizing with a relative biological effectiveness (RBE) of ~1, compared to 1.1 for protons and 3 or more for densely ionizing

radiations such carbon ions and fast neutrons.[15-17] While the mainstay of radiation treatment worldwide is still photon therapy, particle therapy is increasingly prevalent. There are 25 proton therapy centers in operation across the United States with dozens more under construction. Densely ionizing (high RBE) particles are becoming more prevalent as well, with 10 carbon ion centers operational worldwide, and one announced in the US with more expected over the next decade. The main attraction of particle therapy is the ability to improve dose conformality by depositing most of the radiation in the tumor and less radiation in normal tissues[18]. Although protons are more widely available than carbon or other high RBE particles, protons have similar biologic efficacy as standard photon therapy (proton RBE ~ 1.1), but fast neutrons and carbon ions have higher RBE of 3 or more.[16-18] Higher RBE particles have a unique ability to overcome hypoxia, cell cycle radiosensitivity, and other effects that enable some tumors to resist standard photon treatments. For example, *p53* mutated cells, which are associated with higher grade and higher stage bladder cancers[19], are relatively radioresistant to standard photon therapy but more susceptible to high RBE radiation, meaning it is likely that high RBE radiation has a greater impact on the tumor microenvironment and immune milieu[20].

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

Although classical radiation biology states that the cytotoxic effects of radiation on tumor cells are primarily initiated by DNA double strand breaks, there is an emerging understanding of the importance of an "immunogenic cell death" (ICD) as a significant *in vivo* cell death mode. Radiation has been shown to induce all 3 key components of ICD: cell surface translocation of calreticulin to signal dendritic cells, and release of danger signals such as HMGB1 and ATP, which result in priming of CD8+ T-cells[21]. Tumor mutational burden and defects in the DNA repair pathway have been linked to tumor response rate to checkpoint inhibitors such as anti-PD-1 therapy. While bladder cancer is the third highest mutated cancer[22], high RBE particle therapy such as neutrons cause qualitative changes in the sub-cellular distribution and types of DNA damage, and have the potential to shift cells from an apoptotic cell death mode towards the more inflammatory, and possibly immunogenic, mitotic catastrophe and necrotic cell death modes.[23-25] High RBE therapy may cause a more immunogenic tumor microenvironment via release of more tumor neoantigens[26,27]. Mouse total body radiation experiments in the 1970s with neutrons versus standard photons found that the degree of immunosuppression was dependent on the LET of the radiation (neutrons have high LET), and mortality from total body radiation differs based on LET, for the same delivered radiation dose[28].

Immunologic changes in peripheral blood of patients receiving immunotherapy:

Immune profiling of peripheral blood from patients on checkpoint inhibitor immunotherapy has not been widely explored. Huang *et al.* looked at peripheral blood of 29 patients with metastatic melanoma receiving anti-PD-1 antibody pembrolizumab[29]. At 3-weeks into therapy, frequency of Ki67+ CD8 T-cells peaked and then declined, with highest percent of Ki67+ CD8 T-cells around weeks 3-6 after start of pembrolizumab (Ki67+ CD8 T-cells increased from around 7% at baseline to around 14-19% at weeks 3-6 post-pembrolizumab). The increase in Ki67 expression was mostly seen in the PD-1+ versus PD-1 negative CD8 T-cells, with Ki67 increasing from around 9% at baseline to around 23% at 3-weeks (peak response) in the PD-1+ CD8 T-cells population, and non-statistically significant increase in Ki67+ cells in the PD-1 negative CD8 T-cell population (Figure 1). Clinical response was correlated with the fold change of PD-1+ Ki67+ CD8 T-cells after

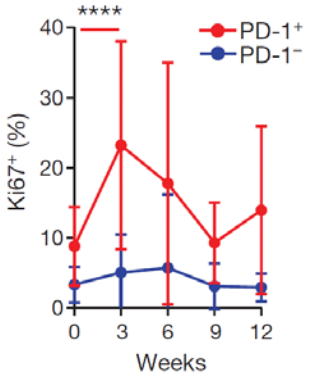

Figure 1. Change in percent Ki67+ CD8 T-cells after start of pembrolizumab therapy (n=29). Red line=PD-1+ cells, blue line=PD-1 negative cells. Adapted from Huang AC et al. Nature 2017 (545):60-65.

anti-PD-1 therapy, adjusted for baseline tumor burden. Patients with longer PFS typically had a low tumor burden and higher fold change in Ki67. Using an arbitrary cutoff of 2.2 fold change in Ki67+ PD-1+ CD8 T-cells after anti-PD-1 therapy at weeks 3-12 compared to baseline, response rate was 40% (8/20) for patients with Ki67 change above the fold, and 29% (2/7) for patients below the fold. T-cell receptor repertoire was compared between pretreatment tumor infiltrating T-cells and peripheral blood CD8 T-cells. Across 6 patients, at peak Ki67 expression after anti-PD-1 treatment, 14 clones were present among the top 10 clones in both tumor and blood, supporting the notion that Ki67+ T-cells in the blood are reinvigorated by anti-PD-1 therapy and contain T-cell clones that are also present in the tumor. This study shows that it is possible to detect immunologic changes in peripheral blood after treatment with anti-PD-1 therapy, and changes in Ki67+ CD8 T-cells may peak around weeks 3-6 and be related to clinical outcome.

Immunologic changes in peripheral blood of patients receiving immunotherapy plus photon radiation:

Twyman-Saint Victor *et al*. reported on 22 patients with metastatic melanoma treated with hypofractionated photon radiation to a single index lesion, followed by 4 cycles of anti-CTLA4 antibody ipilimumab[9]. Radiation was found to increase the diversity of the T-cell receptor repertoire while anti-CTLA4 therapy inhibited T-regulatory (Treg) cells and increased the CD8/Treg ratio. For 10 patients with available pre- and post-treatment blood, two had partial responses in unirradiated tumors and progression-free survival significantly longer than the median. For both of these patients, the percentages of Ki67+ GzmB+ cells increased in PD-1+ Eomes+ CD8 T-cells after treatment (6-12% increase), while the proportion ofPD-1+ Eomes+ T-cells remained at or below the mean (Fig. 2). In contrast, patients with a high percentage of PD-1+ Eomes+ T-cells post-treatment did not have partial responses and had a short progression-free survival, regardless of reinvigoration. Peripheral blood was collected post-radiation treatment and pre-anti-CTLA4 therapy, as well as every 3 weeks with anti-CTLA4 therapy for 4 cycles.

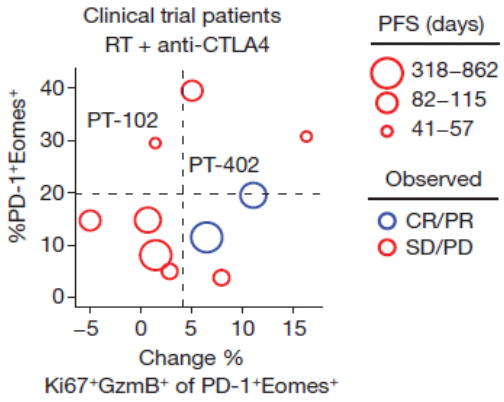

Figure 2. Change in peripheral blood in 10 patients with metastatic melanoma receiving photon radiation to a single index lesion and 4 cycles of anti-CTLA4 therapy. Percentage of Eomes+PD-1+ CD8 T cells in post-treatment blood vs change in%PD-1+Eomes+CD8 T cells that are Ki67+GzmB+ after treatment. Each circle represents a patient. Progression-free survival (PFS) is proportional to circle size and quadrant boundaries are average values for patients under the mean PFS. Concordance index of the random forest model is 0.59. Adapted from Twyman-Saint Victor C et al. Nature 2015 (520):373-377.

Immunologic effects of standard photon radiation on patients receiving immunotherapy for bladder cancer:

Although no clinical data has been published on the effect of standard photon radiation on patients receiving immunotherapy for metastatic bladder cancer, multiple trials are ongoing to test this combination (NCT02826564, NCT02992912, NCT02289209). It is expected that these clinical trials will be looking at immunologic correlates of response as well as overall disease response and survival.

2 Study Objectives

Primary Objective

The primary objective is to assess the overall response rate to neutron radiation (with high relative biological effectiveness relative to standard photon radiation) to a metastatic focus in combination with standard of care checkpoint inhibitor immunotherapy (pembrolizumab), in patients with advanced urothelial carcinoma. Primary endpoint is clinical response as quantified by iRECIST[30]. Response will be assessed on non-radiated, RECIST evaluable lesions. Key iRECIST criteria as detailed by Seymour et al. are detailed below.[30]

Figure 1A-C. Key iRECIST Criteria. Published by Seymour et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. The Lancet Oncology. Mar 2017;18(3):e143-e152.

A	RECIST 1.1	iRECIST
Definitions of measurable and non-measurable disease; numbers and site of target disease	Measurable lesions are ≥10 mm in diameter (≥15 mm for nodal lesions); maximum of five lesions (two per organ); all other disease is considered non-target (must be ≥10 mm in short axis for nodal disease)	No change from RECIST 1.1; however, new lesions are assessed as per RECIST 1.1 but are recorded separately on the case report form (but not included in the sum of lesions for target lesions identified at baseline)
Complete response, partial response, or stable disease	Cannot have met criteria for progression before complete response, partial response, or stable disease	Can have had iUPD (one or more instances), but not iCPD, before iCR, iPR, or iSD
Confirmation of complete response or partial response	Only required for non-randomised trials	As per RECIST 1.1
Confirmation of stable disease	Not required	As per RECIST 1.1
New lesions	Result in progression; recorded but not measured	Results in iUPD but iCPD is only assigned on the basis of this category if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target); the appearance of new lesions when none have previously been recorded, can also confirm iCPD
Independent blinded review and central collection of scans	Recommended in some circumstances—eg, in some trials with progression-based endpoints planned for marketing approval	Collection of scans (but not independent review) recommended for all trials
Confirmation of progression	Not required (unless equivocal)	Required
Consideration of clinical status	Not included in assessment	Clinical stability is considered when deciding whether treatment is continued after iUPD

"i" indicates immune responses assigned using iRECIST. RECIST=Response Evaluation Criteria in Solid Tumours. iUPD=unconfirmed progression. iCPD=confirmed progression. iCR=complete response. iPR=partial response. iSD=stable disease.

Table 1: Comparison of RECIST 1.1 and iRECIST

B	Timepoint response with no previous iUPD in any category	Timepoint response with previous iUPD in any category*
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)
Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified

Target lesions, non-target lesions, and new lesions defined according to RECIST 1.1 principles; if no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for complete response, partial response, and stable disease would be the same. *Previously identified in assessment immediately before this timepoint. "i" indicates immune responses assigned using iRECIST. iCR=complete response. iPR=partial response. iSD=stable disease. iUPD=unconfirmed progression. non-iCR/non-iUPD=criteria for neither CR nor PD have been met. iCPD=confirmed progression. RECIST=Response Evaluation Criteria in Solid Tumours.

Table 2: **Assignment of timepoint response using iRECIST**

C	Timepoint response 1	Timepoint response 2	Timepoint response 3	Timepoint response 4	Timepoint response 5	iBOR
Example 1	iCR	iCR, iPR, iUPD, or NE	iCR, iPR, iUPD, or NE	iUPD	iCPD	iCR
Example 2	iUPD	iPR, iSD, or NE	iCR	iCR, iUPD, or NE	iCR, iPR, iSD, iUPD, iCPD, or NE	iCR
Example 3	iUPD	iPR	iPR, iSD, iUPD, or NE	iPR, iSD, iUPD, NE, or iCPD	iPR, iSD, iUPD, NE, or iCPD	iPR
Example 4	iUPD	iSD or NE	iPR	iPR, iSD, iUPD, or NE	iPR, iSD, iUPD, iCPD, or NE	iPR
Example 5	iUPD	iSD	iSD, iUPD, or NE	iSD, iUPD, iCPD, or NE	iSD, iUPD, iCPD, or NE	iSD
Example 6	iUPD	iCPD	Any	Any	Any	iCPD
Example 7	iUPD	iUPD (no iCPD)	iCPD	Any	Any	iCPD
Example 8	iUPD	NE	NE	NE	NE	iUPD

Eight examples are presented for patients with target disease at baseline, but many more scenarios exist following the same principles. Table assumes a randomised study in which confirmation of complete response or partial response is not required. For patients with non-target disease only at baseline, only iCR or non-complete response or non-progression of disease can be assigned at each timepoint (not shown in the table for ease of presentation). "i" indicates immune responses assigned using iRECIST. iBOR=best overall response. iCR=complete response. iPR=partial response. NE=not evaluable. iUPD=unconfirmed progression. iCPD=confirmed progression. iSD=stable disease. RECIST=Response Evaluation Criteria in Solid Tumours.

Table 3: **Scenarios of assignments of best overall response using iRECIST**

Secondary Objectives:

- Progression free survival.
- Overall survival.
- Safety and tolerability as evaluated by the incidence, severity, duration, causality, seriousness, and type(s) of adverse events as assessed by CTCAE version 4.0

Exploratory Objectives:

- Changes in peripheral blood immune cell subpopulations measured via multi-parameter flow cytometry:
    - Important T-cell subsets using markers such as: CD3/CD8/CD4/Foxp3/CD45RA/CD45RO/CCR7/CD28/CD27/CD57/CD25/HLA-DR/CTLA4/PD-1
    - NK cells will be assessed using CD16/CD56/CD69.
    - B-cells and dendritic cells will be analyzed using: CD19, CD123, CD11c, CD86, MHC class I and II, CD70, and CD54.
    - MDSC will be assessed using: CD11b, CD 14, CD33.
- Next generation sequencing of the T-cell receptor-β locus in genomic DNA from sorted CD4+ and CD8+ T cell subsets from blood samples using the TRB ImmunoSeq kit (Adaptive Biotechnologies).
- Tumor biopsies (pre- and post-treatment of a non-radiated site) in select patients who consent will be assessed for: cell death, tumor infiltrating lymphocytes, expression of cell surface markers including HLA, PDL1, etc., and undergo multiparameter flow cytometry as well as TCR sequencing.

3 Study Design

3.1 General Design

This is a study to test whether densely ionizing, high relative biologic equivalence (RBE) radiation in the form of neutron radiation, can improve the response rate to checkpoint inhibitor immunotherapy in patients with advanced urothelial carcinoma. In this trial, the addition of focal radiation is hypothesized to act as a "vaccine" by releasing tumor neoantigens, with the goal being to augment the immune response. Trial schema is shown below in Figure 1 and study calendar in Table 1. Patients with advanced urothelial carcinoma about to receive checkpoint inhibitor therapy (pembrolizumab), with at least two sites of measurable disease per RECIST 1.1, will undergo neutron radiation to 1-3 sites of disease. At least one site of RECIST 1.1 evaluable disease must remain untreated[31], as only non-radiated sites will serve as index lesions to assess for response rate per iRECIST criteria.[30] Asymptomatic lesions are eligible for radiation as the primary goal of the radiation therapy is to induce an immune response.

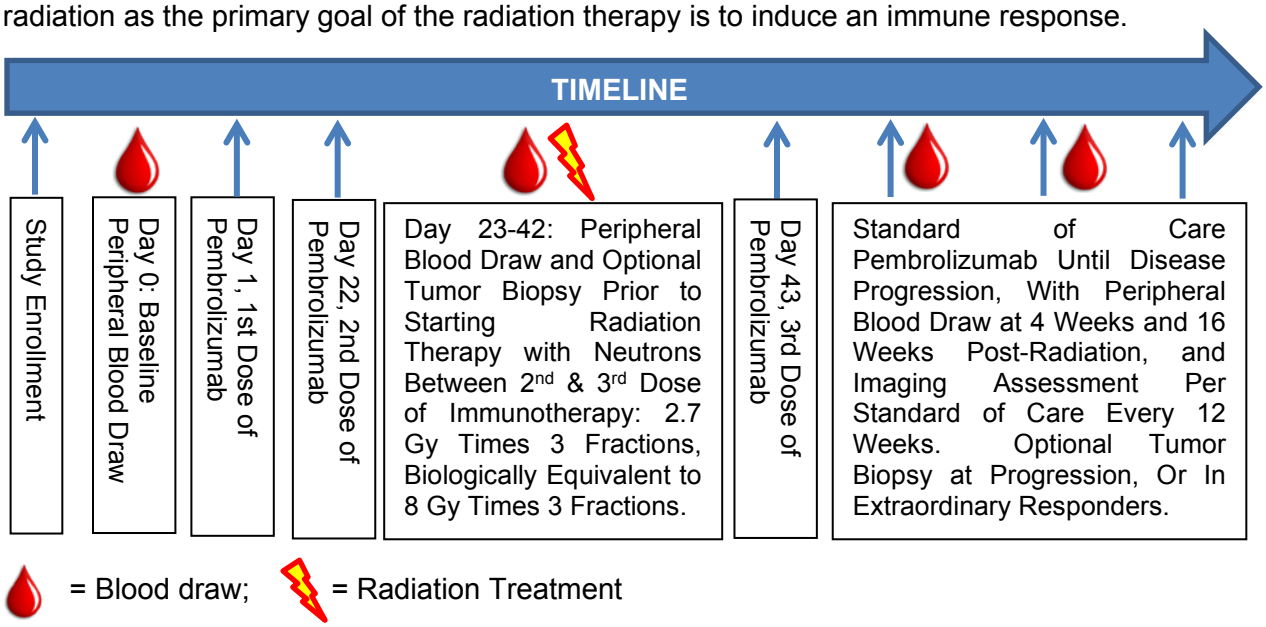

Figure 2. *Clinical trial schema.*

Table 1. Study Calendar.

	Pre-treatment[4]	Between Dose 2 and 3 of Immunotherapy[5]	4-Weeks After End of Radiation[6]	16-Weeks After End of Radiation[7]
Informed Consent	X			
Medical History	X		X	X
Physical Exam	X		X	X
ECOG Performance Status	X		X	X
Blood Draw[1]	X	X	X	X
Radiation Therapy		X		
Tumor biopsy (optional)[2]		X		X
CT C/A/P[3]	X		X	X

[1]Blood draw will consist of 9 tubes: 8 yellow top tubes (BD Vacutainer #364606 Acid citrate dextrose additives ACD Solution A) and 1 red top tube (BD Vacutainer #367820 Clot Activator) to collect and store the following: 1) 6 tubes of 0.5 ml serum; 2) 2 tubes of 1.0 ml plasma; 3) 4 tubes of 0.5 ml PBMC; and 4) 6 tubes of 1 ml PMBC.
[2]Tumor biopsy of a radiated or non-radiated site will be performed for patients who consent.
[3]CT C/A/P is preferred but MRI or PET/CT also acceptable.
[4]All pre-treatment assessments must be performed within 30 days of protocol enrollment, except CT C/A/P which must be within 45 days of protocol enrollment.
Pembrolizumab is typically given every 3 weeks. Dose 2 must happen between 2.5-5 weeks after dose 1, and dose 3 must happen between 6-9 weeks after dose 1.
[6]Acceptable date range is 3 to 6 weeks after radiation ends.
[7]Acceptable date range is 12 to 24 weeks after radiation ends.

Time points for peripheral blood collection are based on prior studies, including Huang *et al.* and Twyman-Saint Victor *et al.* described above[9,29], that show peak changes in peripheral blood immune cell populations around 3-6 weeks post start of therapy for checkpoint inhibitor immunotherapy, and changes after radiation treatment that peak around 2-4 weeks but can persist for months after treatment.

After the study period as outlined above, all patients will be followed at least once every 3 months by the treating oncologist per standard of care. Toxicity, disease response, and survival will be recorded at each clinic visit. Patients are considered off-study once disease progression is detected per iRECIST[30]. Follow up with radiation oncology is not required but records will be obtained from treating oncologist. All correlative studies will be batched for analysis.

3.2. Statistical Plan

This is a single-arm phase 2 study with the primary endpoint of overall response rate per iRECIST. Overall response rate will calculated as the percentage of patients achieving a PR or CR, and will be presented along with the 95% CI. Historical data suggest a response rate of 20%, and we hypothesize a true response rate of 40% with the proposed treatment. With 20 patients, we'll have 80% power to observe an estimated response rate that is statistically significantly higher (at the one-sided significance level of .05) than the fixed rate of 20%. Secondary endpoints overall and progression-free survival will be estimated using the method of Kaplan and Meier with 95% CI obtained using Greenwood estimates of variance. Toxicities as assessed by CTCAE version 4.0 will be summarized as the proportion of patients with such toxicities, in addition to total number of toxicities (allowing for multiple toxicities within a patient) among all patients. The exploratory endpoints, change in peripheral blood cell populations will be assessed by estimating the average change between pre- and post-treatment, and these differences will be calculated for each time point; the one-sample t-test will be used to test the null hypothesis that the change is equal to zero. Trends for changes in differences from pre-treatment to post-treatment across time will be assessed using generalized linear models. Similar methods will be used to assess change in tumor biopsies for patients who consent to such analyses, where changes from pre- to post-treatment will be examined for cell death, tumor-infiltrating lymphocytes and expression of various cell-surface markers.

Should this regimen be deemed to be potentially efficacious as defined above, consideration will be given to conduct a subsequent randomized study designed to compare neutron radiation plus checkpoint inhibitors versus checkpoint inhibitors alone.

3.3. Eligibility Criteria

A. Inclusion Criteria

- Pathologically proven (either histologic or cytologic) diagnosis of urothelial carcinoma
- At least two sites of disease that are measurable by RECIST 1.1 criteria
- Eligible for checkpoint inhibitor immunotherapy (pembrolizumab) per standard of care
- No history of autoimmune disease requiring systemic therapy (e.g. steroids or biologic agents).
- Adequate organ function
    - Hematologic
        - ANC ≥1500 /mcL
        - Platelets ≥ 100,000/mcL
        - Hemoglobin > 9 g/dL
    - Renal
        - Creatinine ≤1.5 x upper limit of normal (ULN) OR ≥60 mL/min
    - Hepatic
        - Total bilirubin ≤1.5 ULN OR direct bilirubin ≤ULN if total bilirubin >1.5 x ULN
        - AST and ALT ≤2.5 x ULN OR <5 x ULN if patient has live metastasis
        - Albumin ≥ 2.5 g/dL
    - Coagulation
        - INR or PT ≤1.5 x ULN unless on anticoagulation therapy, in which case PT or PTT should be in the therapeutic range
        - PTT ≤1.5 x ULN unless on anticoagulation therapy, in which case PT or PTT should be in the therapeutic range

- Eligible for neutron radiation treatment to 1-3 sites of metastatic disease (lesions do not have to be symptomatic)
- No steroids for at least 2 weeks prior to enrollment, and patient must not be expected to require steroids during the study period
- Zubrod Performance Status 0-2
- Age ≥ 18
- Patient must sign study specific informed consent prior to study entry
- Patients who are sexually active must use medically acceptable forms of contraception
- Life expectancy must be > 3 months

B. Exclusion Criteria

- Has a known history of active TB (Bacillus Tuberculosis)
- Hypersensitivity to pembrolizumab or any of its excipients
- Has a known additional malignancy that is progressing or requires active treatment
- Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
- Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
- Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
- Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial
- Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
- Has known history of, or any evidence of active, non-infectious pneumonitis.
- Has an active infection requiring systemic therapy.
- Has received a live vaccine within 30 days of planned start of study therapy.
- Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided there is no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.

4 Study Registration

Subjects will be registered by the FHCRC/UW Study Coordinator and entered into the Protocol Accrual Tracking System (PATS). Information regarding the PATS system is available at http://www.cancerconsortiumorg/rto/protocol_office/pats/. A complete, signed, study consent and HIPAA consent are required for registration.

5 Radiation Therapy

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

5.1 Dose Specifications

Radiation treatments will meet all requirements considered standard clinical care. Patients should ideally be treated with 3 fractions of radiation but up to 5 fractions may be allowed if treating radiation oncologist is concerned with toxicity. All radiation treatments must complete within a 2 week period. Up to 3 sites may be treated concurrently. For neutron radiation, 2.8 Neutron Gy x 3 fractions is the goal dose, but may be decreased to 1.5 Gy x 3-5 fractions at discretion of treating radiation oncologist.

5.2 Localization, simulation, and immobilization

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

5.3 Target Volumes

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

5.4 Critical Structures

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

5.5 Treatment Planning

- Any clinically acceptable, standard of care planning technique may be employed to deliver radiation to the index lesion, including 3D conformal treatment, intensity modulated radiation therapy, and stereotactic radiosurgery. Typically, patients should not be treated with two directly opposed beams due to the volume of tissue receiving high dose radiation, and using at least 3 beams or 2 non-opposed beams is encouraged. All plans are subject to review by the PI. All dose calculations will include corrections for tissue heterogeneities as specified by IROC Houston.

- Dose specifications: at least 95% of the target volume (PTV) is covered by at least 90% of the prescription dose.
- Critical Organ Doses: All standard of care critical organ dose-volume limits will be respected.

5.6 Radiation Quality Assurance Reviews

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

5.7 Radiation Toxicity

Toxicity will be graded based on CTCAE 4.0.

5.8 Criteria for Removal/Withdrawal from Treatment

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

Patients will still receive follow up care per standard of care even if they withdraw from the study. If a subject withdraws consent to participate in the study or aspects of the study, attempts will be made to obtain permission to record at least survival data up to 6 months post-treatment. Details for withdrawal from immunotherapy is detailed below in section 6.1

6 Drug Therapy

Patients will pembrolizumab per standard of care. Prescribing information for pembrolizumab can be found at: https://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf. Dose modification and discontinuation information can be found at: https://www.keytruda.com/static/pdf/guide-for-keytruda.pdf. Standard dosing is 200 mg fixed dose IV infusion every 3 weeks.

6.1 Duration of Therapy

Pembrolizumab therapy will continue until one or more of the following conditions are met:

- The subject withdraws consent.
- Confirmed radiographic disease progression
- Unacceptable adverse experiences
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up
- Administrative reasons

6.2 Pembrolizumab Supportive Care Guidelines

Subjects should receive appropriate supportive care measures per standard clinical care as deemed necessary by the treating oncologist. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the oncologist determines the events to be related to pembrolizumab.

- Pneumonitis:
    - For Grade 2 events, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For Grade 3-4 events, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- Diarrhea/Colitis: Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
    - For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For Grade 2 diarrhea/colitis that persists greater than 3 days, administer oral corticosteroids.
    - For Grade 3 or 4 diarrhea/colitis treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)
    - For T1DM or Grade 3-4 Hyperglycemia
        - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
        - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- Hypophysitis:
    - For Grade 2 events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4

weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
    - For Grade 3-4 events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- Hyperthyroidism or Hypothyroidism: Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.
    - Grade 2 hyperthyroidism events (and Grade 2-4 hypothyroidism):
        - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
        - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
    - Grade 3-4 hyperthyroidism
        - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- Hepatic:
    - For Grade 2 events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
        - Treat with IV or oral corticosteroids
    - For Grade 3-4 events, treat with intravenous corticosteroids for 24 to 48 hours.
    - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

- Renal Failure or Nephritis:
    - For Grade 2 events, treat with corticosteroids.
    - For Grade 3-4 events, treat with systemic corticosteroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

- Management of Infusion Reactions: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 2 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 2. Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated;	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable	None

intervention not indicated	in the opinion of the oncologist.	
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDS • Acetaminophen • Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the oncologist. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (±30 minutes) prior to infusion of pembrolizumab with: • Diphenhydramine 50 mg po (or equivalent dose of antihistamine). • Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilator support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDS • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids • Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the oncologist. Hospitalization may be indicated. **Subject is permanently**	No subsequent dosing

	discontinued from further trial treatment administration.	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

7 Adverse Event Reporting Requirements

7.1 Adverse Event (AE) Reporting

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

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

7.2 Expected Toxicities

Pembrolizumab will be given per standard of care and toxicity information is available from phase III data. Full prescribing information for pembrolizumab can be found at: https://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf.

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

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

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

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

8 Data and Safety Monitoring Plan

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

8.1 Early Stopping Rules

Early stopping of this trial will be any grade 5 adverse events (AEs) or multiple (2 or more) grade 4 AEs occurring within ≤ 30 days after the end of treatment defined as possibly, probably, or definitely related to radiation treatment (per CTCAE, v.4.0). All AE's grade 4 or higher must be reported to the PI within 24 hours. All grade 3 AEs must be reported to the PI within 48 hours

8.2 Interim Data Review

Interim reports with statistical analyses will be prepared twice per year until the initial treatment results have been presented or published. In general, the interim reports will contain the following information:

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

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

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

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

9 Data Management/Confidentiality

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

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

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