NCT #: NCT03276572

TITLE: Phase I dose−escalation trial of ^{225}Ac−J591 in patients with metastatic castration−resistant prostate cancer

IRB Protocol #: 1706018281
IND/IDE # 135267
Version Date: August 30, 2021

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Confidentiality Statement

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List of Abbreviations

^{225}Ac	Actinium−225
ADT	Androgen Deprivation Therapy
AE	Adverse Event
AR	Androgen Receptor
CFR	Code of Federal Regulations
CRF	Case Report Form
CRPC	Castration Resistant Prostate Cancer
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTSC	Clinical Translational Science Center
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
^{68}Ga	Gallium−68
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
HRBAF	Human Research Billing Analysis Form
ICF	Informed Consent Form
IND	Investigational New Drug
IRB	Institutional Review Board
^{177}Lu	Lutetium−177
mCRPC	Metastatic Castration Resistant Prostate Cancer
MTD	Maximum tolerated dose
NA	Not Applicable
NCI	National Cancer Institute
PC	Prostate Cancer
PHI	Protected Health Information
PI	Principal Investigator
PSMA	Prostate Specific Membrane Antigen
^{223}Ra	Radium−223
REDCap	Research Electronic Data Capture
RP2D	Recommended Phase 2 dose
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction

UAP	Unanticipated Problem
WCM	Weill Cornell Medicine

Protocol Summary

Full Title:	Phase I dose−escalation trial of ^{225}Ac−J591 in patients with metastatic castration−resistant prostate cancer
Short Title:	^{225}Ac−J591for mCRPC
Clinical Phase:	Phase I
Principal Investigator:	Scott T. Tagawa, MD, MS
Sample Size:	*N*= 2 − 52 (accelerated dose−escalation study design up to 7 dose−escalation cohorts)
Accrual Ceiling:	This study will enroll up to 52 subjects who receive treatment.
Study Population:	Adult male patients of ≥18 years age with documented progressive metastatic CRPC
Accrual Period:	3 years (approximately 1−2 patients per month).
Study Design:	Phase I dose escalation study with ^{225}Ac−J591 using single dose regimen will be performed in patients with documented progressive metastatic CRPC. The cumulative ^{225}Ac dose [13.3 KBq/Kg – 93.3 KBq/Kg or 0.36 μCi/Kg – 2.52 μCi/Kg] will be escalated in up to 7 different dose levels (accelerated dose−escalation study design).
Study Duration:	Approximately 3 months after enrollment, then transition to long−term follow up
Study Agent:	^{225}Ac−J591, single dose, IV administration
Primary Objectives:	• Determine the dose limiting toxicity (DLT) of single dose of ^{225}Ac−J591 • Determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of ^{225}Ac−J591 in a single dose regimen
Secondary Objectives:	• To assess the proportion with PSA decline following single dose of ^{225}Ac−J591 • To assess radiographic response rate by PCWG3 criteria • To assess biochemical and radiographic progression−free survival by PCWG3 criteria • To assess overall survival following single dose of ^{225}Ac−J591 • To assess safety of single dose ^{225}Ac−J591 as assessed by CTCAE 4 • To assess changes in CTC count as measured by CellSearch and the proportion with favorable and undetectable CTC count and LDH at 12 weeks following single dose ^{225}Ac−J591 • To assess patient reported outcomes using FACT−P and the Brief Pain Inventory short form
Exploratory Objectives:	• Disease assessment with PSMA−ligand based imaging prior to and following investigational treatment • To assess genomic alterations in relationship to outcome following fractionated dose ^{225}Ac−J591

- To assess immune effects of ^{225}Ac-J591
- To assess reproducibility of ^{68}Ga-PSMA-HBEC-CC PET/CT

Endpoints: Dose limiting toxicity (DLT), adverse event rate, Maximum tolerated dose (MTD), recommended phase II dose, response rate, and progression free survival.

Schema

Screening: Written informed consent, history, physical examination, conventional imaging modalities (CT/MRI, Bone scan**),** PSMA−ligand based PET/CT imaging

↓

Treatment visit: Injection of single dose of ^{225}Ac−J591

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F/U visits: Adverse events, concomitant medications/procedures, PSA and LDH levels

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Efficacy Evaluation visit: Adverse events, concomitant medications/procedures, PSA, LDH and Testosterone levels, CTC count, CT/MRI, bone scan, PSMA−ligand based PET/CT imaging

↓

Short−term and Long−term Follow up

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1. STUDY OBJECTIVES

1.1 Primary Objectives

To define the dose limiting toxicity (DLT) and maximum tolerated dose (MTD) as well as to determine the recommended phase II dose of ^{225}Ac−J591 in a single dose regimen for patients with progressive metastatic castration−resistant prostate cancer.

1.2 Secondary Objectives

- To assess the proportion with PSA decline following single dose of ^{225}Ac−J591
- To assess radiographic response rate by PCWG3 criteria
- To assess biochemical and radiographic progression−free survival by PCWG3 criteria
- To assess overall survival following single dose of ^{225}Ac−J591
- To assess safety of single dose ^{225}Ac−J591 as assessed by CTCAE 4
- To assess changes in CTC count as measured by CellSearch and the proportion with favorable and undetectable CTC count and LDH at 12 weeks following single dose ^{225}Ac−J591
- To assess patient reported outcomes using FACT−P and the Brief Pain Inventory short form

1.3 Exploratory Objectives

- Disease assessment with PSMA−ligand based imaging prior to and following investigational treatment
- To assess genomic alterations in relationship to outcome following fractionated dose ^{225}Ac−J591
- To assess immune effects of ^{225}Ac-J591

2. BACKGROUND

2.1 Disease

Prostate cancer (PC) is a significant health burden, with 180,890 new diagnoses and 26,120 deaths in the United States in 2016(1). Despite advances in diagnostic technology and treatment strategies, up to 40% of patients treated with primary therapy with curative intent will experience disease progression. PC deaths are typically the result of metastatic castration−resistant prostate cancer (mCRPC), and historically the median survival for men with mCRPC has been less than two years (2). The recent availability of novel treatments for mCRPC has given a resurgence of hope for these men as studies now demonstrate improved survival with a variety of new agents. However, the unfortunate reality is that mCRPC remains an incurable disease, and it is against this backdrop that we look to the future with cautious optimism and new hope for scientific discovery.

First line therapy for advanced PC is androgen deprivation with a mean duration of efficacy of 12−18 months, although there is a wide variation in response in this heterogeneous disease. Upon progression, the disease becomes castration−resistant and subsequently many such patients

develop frank metastasis. Exact mechanism of transformation from castration−sensitive prostate cancer to castration−resistant disease is still not fully understood, but with recent scientific innovations in basic research, there is now a better understanding. Now we know that despite castrate levels of androgens, the androgen receptor (AR) remains active and continues to drive prostate cancer progression (3, 4). This understanding has led to the development of novel agents aimed at further decreasing androgen production or blocking AR function. However, there are also many other biologic pathways that function independent of androgen signaling resulting in CRPC. With a greater understanding of the tumor biology, there is hope for continued development of innovative treatment options that improve survival for men with mCRPC.

Treatment of mCRPC has drastically changed over the past decade. Metastatic castration−resistant prostate cancer (mCRPC) poses a particular clinical challenge in need of additional therapeutic approaches beyond classic androgen deprivation therapies. Currently, the chemotherapy compounds docetaxel and cabazitaxel, the androgen receptor signaling inhibitor enzalutamide, CYP−17−inhibitor abiraterone, autologous cellular immunotherapy with sipuleucel−T, and the bone−seeking α−emitter ^{223}Ra have shown improved overall survival (OS) and most have demonstrated quality of life advantages as well (5-13). These agents have been tested in multiple "disease states" of CRPC to determine if or when patients might benefit from each treatment. In all cases, however, these now established therapies become ineffective in controlling tumor progression over time(14). Novel therapies are urgently needed in order to further ameliorate the course of the disease. Other treatments for men with mCRPC have been shown to improve outcomes, but remain to be approved by the FDA (15).

2.2 PSMA

In PC, the most well established, prostate−restricted, cell surface antigen yet identified is prostate specific membrane antigen (PSMA)(16-20). PSMA is a trans−membrane protein with a 707−amino−acid extracellular portion. The PSMA gene (FOLH1) is located on the short arm of chromosome 11.

Although first thought to be entirely prostate−specific, subsequent studies have demonstrated that PSMA is also expressed by cells of the small intestine, proximal renal tubules and salivary glands (20). PSMA is expressed in the apical region of normal prostatic cells, the epithelium surrounding prostatic ducts(21). Dysplastic changes in the prostate result in the expression of PSMA on the luminal surface of prostatic ducts (22, 23). Increasing prostate cancer stage and grade result in higher cell membrane PSMA expression (24, 25). The eventual progression to advanced prostate cancer and castrate resistance corresponds to further increases in PSMA expression (26). PSMA expression in prostate cancer cell membranes is 100− to 1000−fold that in normal cells (24, 25). Thus, PSMA represents a promising target for imaging and therapy of prostate cancer.

2.3 Anti−PSMA Monoclonal humanized antibody (J591)

J591 is a de−immunized monoclonal antibody directed at the extracellular domain of human PSMA. Initially J591 was developed as a mouse antibody by Dr. Neil Bander's lab at Weill Cornell

Medical Center. Humanized J591 was derived from murine J591 using Biovation's DeImmunization technology (Biovation, Aberdeen, Scotland, UK). In this technology, individual amino acids in predicted B and T cell epitopes were replaced with alternative amino acids such that the murine epitopes are no longer immunogenic to the human immune system.(27) The modification results in a non−immunogenic antibody that may be administered to patients on multiple occasions and over long periods without inducing an immune response. J591 is produced from NS0 cells by Lonza (Lonza Biologics, Slough, UK).

The humanized version of J591 has provided promising results in imaging both localized PC and metastatic disease. Initial phase I/II studies using J591 trace−labeled with ^{111}Indium (^{111}In) and ^{177}Lutitium (^{177}Lu) using a DOTA chelate showed that repetitive dosing was well tolerated without the development of a human anti−humanized antibody (HAHA) response (28-33). No dose limiting toxicity occurred at imaging doses of radionuclide conjugates and the maximum tolerated dose was not reached. Excellent tumor targeting could be detected at all dose levels of the mAb. No mAb targeting to sites other than those involved by PC was observed although, as seen in other trials using radiometals, the liver is the primary site of radiometal metabolism. Percent injected dose in the liver diminished with increasing dose of cold J591, and higher doses were associated with longer plasma clearance times (34-36). The future efforts are to explore further therapeutic options by radiolabeling J591 with more cytotoxic radionuclides like α– particle emitter Actenium−225 that holds the potential to induce a much stronger anti−tumor response.

2.3.1 Prior clinical experience with J591 and radiolabeled−J591 in men with prostate cancer:

2.3.1.1 Pilot study with huJ591

Initial phase I studies using huJ591 trace−labeled with ^{111}In using a DOTA chelate showed that repetitive dosing was well tolerated with total doses of up to 500 mg/m^2 without the development of a human anti−humanized (de−immunized) antibody (HAHA) response(37). No dose limiting toxicity occurred and the maximum tolerated dose was not reached. Excellent tumor targeting could be detected at all dose levels of mAb. No mAb targeting to non−prostate cancer sites was observed although, as seen in other trials using radiometals, the liver is the primary site of excretion.

2.3.1.2 Phase I and II studies with single dose radiolabeled J591

Two independent phase I clinical trials were initally performed at WCMC using a single−dose of ^{177}Lu or ^{90}Y linked via a DOTA chelate to huJ591 in subjects with metastatic, hormone−refractory prostate cancer (28, 31). The primary objectives of these trials were to define the maximum tolerated doses (MTD) of the isotopes as well as to further define dosimetry, pharmakokinetics, and HAHA of the radiolabeled mAb conjugates. Anti−tumor responses were assessed as a secondary endpoint. The design and entry criteria of the 2 trials were identical. Eligible subjects had a prior histologic diagnosis of prostate cancer and evidence of progressing, recurrent or

metastatic disease defined by at least 3 serially rising PSAs and/or radiographic studies. As prior studies had demonstrated that all prostate cancers were PSMA−positive (38), no determination of PSMA expression was done.

i. Phase I Trial of ^{177}Lutetium−Labeled J591 in subjects with metastatic castration−resistant prostate cancer (CRPC)(28):

^{177}Lu−J591 was evaluated in patients with metastatic CRPC demonstrating acceptable toxicity (MTD=70 mCi/m^2), excellent targeting of metastatic sites and biologic activity. Thirty−five subjects received ^{177}Lu−J591, of whom 16 received up to three doses. Myelosuppression was dose limiting at 75 mCi/m^2, and the 70 mCi/m^2 dose level was determined to be the single−dose MTD. Repeat dosing at 45 to 60 mCi/m^2 was associated with dose−limiting myelosuppression; however, up to three doses of 30 mCi/m^2 could be safely administered. Nonhematologic toxicity was not dose limiting. Targeting of all known sites of bone and soft tissue metastases was seen in all 30 subjects with positive bone scan, computed tomography, or magnetic resonance images. No subject developed a human anti−J591 antibody response to deimmunized J591 regardless of number of doses. Biologic activity was seen with four subjects experiencing ≥50% declines in PSA levels lasting from 3+ to 8 months. An additional 16 subjects (46%) experienced PSA stabilization for a median of 60 days (range, 1 to 21+ months).

ii. Phase 2 trial of ^{177}Lutetium (^{177}Lu) radiolabeled J591 (^{177}Lu−J591) in subjects with metastatic castration−resistant prostate cancer (CRPC) (39):

In a phase II trial patients with progressive metastatic CRPC received a single dose of ^{177}Lu−J591 in two cohorts (65 and 70 mCi/m^2). Cohort 1: 15 patients; Cohort 2: 17 patients. The primary endpoint was PSA and measurable disease response assessed at week 12 and the secondary endpoint was to evaluate toxicity. One ^{177}Lu imaging study was done at 1 week post−treatment to confirm tumor targeting. Three patients achieved PSA declines of >50%; 31% had at least 30% decrease in PSA (the cutoff associated with survival benefit in chemotherapy trials). Though the phase I MTD was 70 mCi/m^2, based upon FDA restrictions, the initial cohort was treated at 65 mCi/m^2. In an exploratory analysis, there was a dose−response relationship. The ≥30% PSA response rates in the 65 mCi/m^{2}and 70 mCi/m^2 cohorts were 13% and 47% respectively (p=0.06); and any PSA decrease in 46% vs. 71% respectively. Hematological toxicity was similar to that in phase I trials; no significant drug−related non−heme toxicity occurred. Grade (Gr) 4 thrombocytopenia occurred in 42% and 9 patients received platelet transfusions. Targeting of known sites of PC metastasis was observed in 30 of 32 (94%) patients.

iii. Phase I trial of fractionated dose ^{177}Lu−J591 in men with metastatic castration−resistant prostate cancer (40, 41):

Men with progressive metastatic CRPC received 2 fractionated doses two weeks apart. Initially, 6 cohorts of 3−6 pts got 2 doses of ^{177}Lu−J591 2 wks apart (20 mCi/m2, escalating to 45 mCi/m2 x2). Subsequently, pts enrolled in 2 expansion cohorts at the recommended phase 2 doses (RP2D). Planar ^{177}Lu−J591 imaging was semi−quantitatively scored. The endpoints were PSA changes and

survival (OS); as well as CTC count (CellSearch) changes in the expansion cohorts. 49 patients, with median age 74.1 years (range 55−95), median PSA of 44.9 ng/mL (1.9−766.5); 83.7% with bone, 61.2% with lymph node, 40.8% with visceral metastasis. 8.2% were CALGB (Halabi) low, 34.7% were intermediate, 57.1% were in high−risk group. RP2D's of fractionated ^{177}Lu−J591 were 40 mCi/m2 x2 or 45 mCi/m2 x2 with option for GCSF. PSA changes for the low dose group were reported as 6.3% showing >50% PSA decline, 12.5% reporting >30% PSA decline, and 37.5% with any PSA decline. within RP2D group 21.2% showing >50% PSA decline, 42.4% reporting >30% PSA decline, and 66.7% with any PSA decline. The median overall survival for low dose group was 14.6 months and for RP2D group was 27.7 months. Accurate targeting of ^{177}Lu−J591 was seen in 79.6%. Patients with lower PSMA expression by imaging were less likely to respond (p=0.07). Of 25 with CTC counts, 14 declined, 8 stably favorable, and 3 increased. RP2D was associated with more PSA declines (p=0.036) and longer OS (p=0.004), even after controlling for CALGB prognostic grouping (adjusted HR 0.42 [95% CI 0.21, 0.84] p=0.01). Predictable, reversible myelosuppression was seen. 36 (73.5%) patients had grade 3/4 hematologic toxicities; 19 (57.6%) had Grade 4 hematologic toxicities in RP2D cohorts with 45.4% receiving prophylactic platelet transfusions (median 1, range 1−4) and 6 GCSF. 14 (28.6%) had infusion reactions (without pre−meds), with 1 patient having Grade 2 infusion reaction leading to withdrawing from the study prior to his 2nd dose. 5 (10.2%) had transient Gr 1/2 AST/ALT. This study concluded that fractionated ^{177}Lu−J591 is well tolerated with predictable, reversible myelosuppression and PSA and CTC declines. Additionally, with dose−fractionation, the cumulative dose MTD is 14−28% higher than single dose MTD with similar toxicity.

Table 1: Toxicities data from Phase 2 single dose ^{177}Lu−J591 Study and Phase 1 fractionated ^{177}Lu−J591 study:

	Single Dose		Fractionated Dose		
Cumulative Dose (mCi/m^2)	65	**70**	**70** (35 + 35)	80 (40 + 40)	90 (45 + 45)
Grade 4 Thrombocytopenia	27%	56.3%	40%	50%	58.8%
Platelet Transfusion	7%	41%	0%	31.3%	52.9%
Grade 4 Neutropenia	0%	37.5%	0%	31.3%	29.4%
Febrile Neutropenia	0%	2.1%	0%	0%	5.8%

In addition, fractionated dosing allowed concurrent dosing with myelosuppressive chemotherapy:

iv. Phase I trial of fractionated dose ^{177}Lu−J591 plus docetaxel/prednisone in men with metastatic castration−resistant prostate cancer (42):

Following progression on primary hormonal therapy, chemotherapy can offer symptomatic improvement as well as incremental survival benefit. However, responses are transient and all men eventually suffer from progression of disease as described above with single−agent anti−PSMA based radioimmunotherapy. The combination of taxane chemotherapy with radiotherapy has been used in several diseases because of the radiosensitizing effects of taxane−based chemotherapy. In addition to favorable results from fractionated RIT and the radiosensitizing effects of taxane−based chemotherapy, it is hypothesized that the additional debulking by chemotherapy will overcome some of the limits imposed by the physical characteristics of ^{177}Lu. Based upon this theory, a phase I trial of docetaxel and prednisone with escalating doses of fractionated ^{177}Lu−J591 was initiated. 15 men with median age 69.1 (49.3−80.8) were enrolled. The MTD/RP2D of ^{177}Lu−J591 was 40 mCi/m^{2}x2 doses (delivered with cycle 3 of docetaxel), with 73.3% showing >50% PSA decline, 80.0% reporting >30% PSA decline, and 86.7% with any PSA decline. Predictable, reversible myelosuppression was seen. Even at the highest dose level, no dose limiting toxicity was observed, with short−term / reversible grade 4 neutropenia in 33% and grade 4 thrombocytopenia in 13%.

2.4 Alpha particles

Radionuclides that emit alpha particles (helium nucleus consisting of 2 protons and 2 neutrons) deliver about 4,000−fold the energy of a beta particle (composed of an electron or positron). While thousands of beta particle hits are required to kill a cell, a single alpha hit is sufficient to kill by causing irreparable double strand DNA breaks. In addition to their high energy, the range of emission is only 50−100μ leading to less potential for toxicity to bystander cells. Based upon strong early studies, an international phase 3 study led by co−investigator Dr. Sartor demonstrated an overall survival benefit in addition to an improvement in patient reported outcomes, leading to FDA approval (13, 43). Importantly, as predicted based upon the short path length, the toxicity profile of ^{223}Ra (Radium−223) is favorable with only mild myelosuppression and gastrointestinal adverse events. Very clearly, this established α−emitting radiopharmaceutical in the clinic. However, ^{223}Ra is "targeted" simply because it chemically resembles calcium and is, therefore, preferentially deposited in sites of active bone formation. Because the bone−targeting radiopharmaceutical anti−tumor effect derives from accumulation in proximity to malignant cells and/or stroma, these agents entirely ignore soft tissue and extra−osseous visceral metastases. As such, a method of delivering an α−emitting particle directly to tumor cells would be a significant advance.

Converting indirect bone targeting to direct tumor cell targeting via a PSMA−specific mAb will result in the alpha particle being internalized and deposited adjacent to the nucleus. Such proximity to the nucleus (and the DNA) yields even greater potency. In addition, unlike the ^{223}Ra indirect approach, direct tumor targeting would enable treatment of PC outside the bone (e.g., in the soft tissue and circulation) and allow killing of bone marrow micro−metastases before they induce the increased bone turnover necessary for successful use of ^{223}Ra. We expect that by targeting macro− and microscopic metastatic tumor sites with PSMA−targeted alpha particles, we will be able to demonstrate even greater efficacy than seen with β−emitters, potentially with less toxicity. There have been anecdotal reports of PSMA−targeted α−particles utilizing actinium−225 (^{225}Ac) in

Europe, including a recent publication with impressive results(44). In addition, there are unpublished reports of "success" with PSA declines and scan improvements, with issues of impaired quality of life due to salivary gland toxicity. However, like the experience with PSMA−targeted β−radiation, there are no prospective trials. Based upon the high potency of α−particles, it may be even more important to target tumor rather than the other sites of low−levels of PSMA expression such as proximal renal tubules, salivary glands, and small bowel. In fact, a recent published report demonstrated significant proximal renal tubule uptake and late radiation nephropathy in mice models(45). Therefore, mAb−based α−particle delivery should be advantageous with less expected myelosuppression than seen with β−emitters. We have demonstrated the ability to deliver radioactive particles to both known (macroscopic) and unknown (micrometastatic, **Figure 1**) sites of disease (28, 31, 32, 39, 41, 46-56). Interestingly, but not surprisingly, in the study of 12 doses of ^{223}Ra, there was good control of disease in the bone, but progression occurred in extraosseous sites of disease. There is essentially no resistance mechanism to the high energy of α−emitters provided the short−range particle can be delivered into PC cells in close proximity to the nucleus.

2.4.1 Actinium−225 for Radioimmunotherapy:

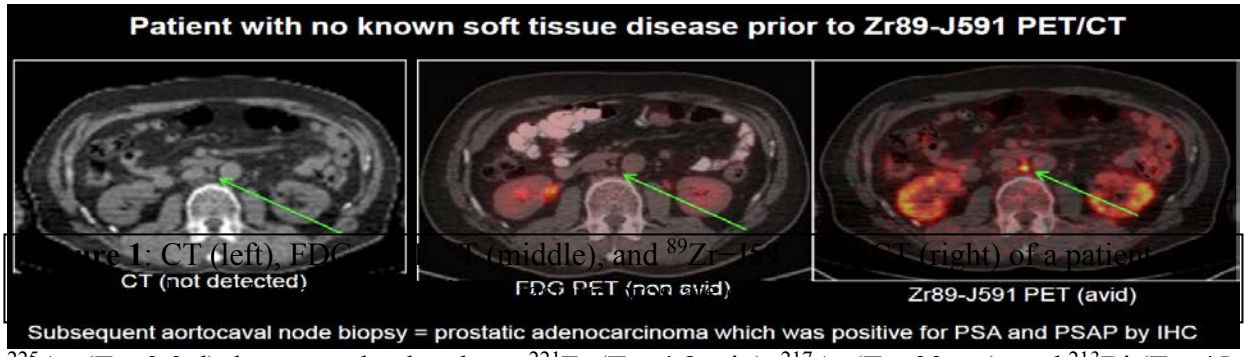

Figure 1: CT (left), FDG PET (middle), and ^{89}Zr−J591 PET/CT (right) of a patient

^{225}Ac ($T_{½}$, 9.9 d) decays to the daughters ^{221}Fr ($T_{½}$, 4.8 min), ^{217}At ($T_{½}$, 33 ms), and ^{213}Bi ($T_{½}$, 45.6 min)(57). Since each of these nuclides disintegrates with emission of one α−particle, a total of 4 α particles are emitted and deliver massive damage to the target cell. In order to ensure the energy is deposited within the cell, internalization of the Ac−225 into the tumor cells is desired.

For PSMA−targeted therapy, the advantage of J591 mAb as an internalizing carrier molecule was previously demonstrated almost 2 decades ago(58). Early work with ^{225}Ac labeled mAbs was limited by difficulty of preparing a very stable ^{225}Ac−chelator−antibody complex. Recently, an efficient, 1−step radiolabeling method that produces high specific activity, stable, therapeutically active ^{225}Ac−DOTA−mAb complex was developed(56). As a result, it is now possible to design an optimal clinical protocol to study the potential clinical utility of ^{225}Ac−DOTA−J591 mAb for the treatment of mCRPC.

2.4.2 Clinical studies:

From a recent news publication by Joint Research Center (JRC), European Commission (59), 80 patients have been treated over a three−year period. As per the report, several patients had a longer response, one of them being without relapse/progression for more than 2 years. Patients received a dose of 100 kBq/kg at bimonthly intervals, considered to be safe and effective with the only side effect being xerostomia. Most patients had received multiple lines of treatment prior to the investigational therapy. The expected median survival is 2−4 months. After treatment, 75% of the study population was alive at 24 weeks and 75% patients had a decline in tumor size and serum PSA. The same anecdotal reports describe toxicity from small−molecule ligand PSMA targeting particularly salivary glands clinically with the potential for renal toxicity as well) that is not present with mAb targeting

A case report of 2 patients with mCRPC who received 100 KBq/Kg of 225Ac−PSMA−617 bimonthly has been published (44). One patient was treated with 3 cycles of 9–10 MBq (100 kBq per kilogram of body weight) of 225Ac−PSMA−617 at bimonthly intervals. The patient received an additional 6 MBq of 225Ac−PSMA−617 as consolidation therapy atleast 2 months later and his PSA dropped to 0.1 ng/mL (from >3000 ng/mL at screening). Additionally, all previously PSMA−positive lesions on scans had visually disappeared on PSMA PET/CT. The second patient was considered resistant to 177Lu−PSMA−617 after 2 cycles and was instead offered 225Ac−PSMA−617. He received 3 cycles of 6.4 MBq (100 kBq/kg) at bimonthly intervals. His PSA declined from 419 ng/mL to <0.1 ng/mL, along with a complete remission on PSMA PET/CT. Only toxicity reported was xerostomia.

Based on an initial ^{89}Zr−J591 mAb pilot PET/CT imaging study in 10 patients with PC, the radiation dosimetry for ^{225}Ac−J591 mAb was estimated assuming an organ weighting factor of 5(55). Since then we have completed additional clinical studies with ^{89}Zr−J591 (56, 60).[61,64] Data provided by McDevitt et al. is shown in **Table−2**. It appears that the dose−limiting organs for RIT are red marrow followed by liver, kidney and lung. Assuming the dose limit for red marrow is 2 Gy, the maximum activity that can be administered is about 6.06 MBq or 160 μCi.

Table 2: ^{225}Ac−J591 Dosimetry

Organ	Dose limit	Dose	Activity limit*	
	Gy	mGy/MBq	MBq	mCi
Red Marrow	2	66	6.06	0.16
Kidney	23	382	12.04	0.33
Liver	40	591	13.54	0.37
Lung	20	79	50.93	1.38
* Activity limits estimated with weighting factor = 5				

2.5 Radionuclide−chelating PSMA ligand

^{68}Ga−PSMA−HBED−CC (^{68}Ga−PSMA−11)

PSMA has recently been discovered as a promising target for radioisotope based approaches for PET imaging, for example, using ^{68}Ga−labeled PSMA ligands(61-66). All of these trials report retrospective data on case reports/case series. No well−designed prospective clinical trial has been published/reported yet.

During the last two decades, many efforts have been undertaken to develop PSMA−ligands(67, 68). One of these ligands, the small molecule Glu−NH−CO−NH−Lys−(Ahx)−[^{68}Ga(HBED−CC)], also known as PSMA−11®, PSMAHBED, Glu−CO−Lys(Ahx)−HBED−CC, DKFZ−PSMA−11, PSMA−HBED− CC, PSMA−HBED, PSMA or Prostamedix™, developed at the German Cancer Research Center Heidelberg (DKFZ), has become the most clinically used radiotracer. This compound shows a strong binding affinity to PSMA as well as a highly efficient internalization into PCa cells(69, 70). PET/CT−imaging with ^{68}Ga−PSMA−11 has demonstrated this novel method as an important imaging modality for diagnosing recurrent PCa(61, 71-73). Perera et al., did systematic review of ^{68}Ga−PET articles and Sixteen articles involving 1309 patients were analyzed. On per−patient analysis, the summary sensitivity and specificity were both 86%. On per lesion analysis, the summary sensitivity and specificity were 80% and 97%, respectively(74).

[redacted] produces PSMA−HBED−CC for clinical use. Upon purchase and shipment to Weill Cornell Medicine, the peptide will be labeled with ^{68}Ga with the final product being ^{68}Ga−PSMA−HBED−CC. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine.

The material produced are subjected to quality assurance testing as outlined in the Food and Drug Administration "Points to Consider". All production data and supporting documents, together with the results of all quality assurance testing, have been provided to and reviewed by the Food and Drug Administration (^{68}Ga−PSMA−HBED−CC, IND# 124495).

Results from selective clinical trials using ^{68}Ga−PSMA−HBED−CC to evaluate its diagnositic value are reported here under:

i. **Diagnostic value of ^{68}Ga−PSMA−HBED−CC PET/CT imaging in the diagnosis of recurrent prostate cancer**(72):

The study reports data from a retrospective analysis of 319 patients who underwent ^{68}Ga−PSMA−ligand PET/CT from 2011 to 2014. The ^{68}Ga−PSMA−HBED−CC solution was applied to the respective patient via an intravenous bolus injection (mean of 172.4 MBq ±70.9, range 40 – 400 MBq, median 161 MBq). A non−contrast−enhanced CT scan was performed 1−hour post tracer injection. Histological verification was performed in 42 patients after the ^{68}Ga−PSMA−ligand PET/CT. Tracer uptake was measured in 901 representative tumor lesions. 82.8% of the patients had at least one lesion indicative of PCa was detected. Tumor−detection was

positively associated with PSA level and Androgen deprivation therapy (ADT). Gelason Score and PSA doubling time (PSA−DT) were not associated with tumor−detection. The average maximum standardized uptake value (SUVmax) of tumor lesions was 13.3 ± 14.6 (0.7−122.5). Amongst lesions investigated by histology, 30 were false−negative in 4 different patients, and all other lesions (n = 416) were true−positive or true−negative. A lesion−based analysis of sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) revealed values of 76.6%, 100%, 91.4% and 100%. A patient−based analysis revealed a sensitivity of 88.1%.

ii. **PET imaging with a ^{68}Ga−PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumor lesions** (75):

Initial clinical studies with the ^{68}Ga−labeled PSMA−HBED−CC were conducted at Heidelberg University Hospital and the German Cancer Research Center to assess the biodistribution of ^{68}Ga−PSMA−HBED−CC in normal tissues and tumor lesions. A total of 37 patients with prostate cancer and rising PSA levels were subjected to ^{68}Ga−PSMA−HBED−CC PET/CT imaging. Quantitative assessment of tracer uptake was performed 1 and 3 h post−injection by analysis of mean and maximum standardized uptake values (SUVmean/max) of several organs and 65 tumor lesions. Subsequently, tumor to background ratios were calculated.

The ^{68}Ga−PSMA−HBED−CC PET/CT images showed intense tracer uptake in both kidneys and salivary glands. Moderate uptake was seen in lacrimal glands, liver, spleen and in small and large bowel. Quantitative assessment revealed excellent contrast between tumor lesions and most normal tissues. Of 37 patients, 31 (83.8 %) showed at least one lesion suspicious for cancer at a detection rate of 60 % at PSA <2.2 ng/ml and 100 % at PSA >2.2 ng/ml. Median tumor to background ratios were 18.8 (2.4−158.3) in early images and 28.3 (2.9−224.0) in late images. Within healthy organs, kidneys and salivary glands demonstrated the highest radiotracer uptake. Lesions suspicious for prostate cancer presented with excellent contrast as early as 1−hour after injection with high detection rates even at low PSA levels.

iii. **^{68}Ga−labeled PSMA ligand as superior PET tracer for the diagnosis of prostate cancer: Comparison with ^{18}F−FECH** (63):

This study was also published by the Heidelberg group, compared ^{68}Ga−PSMA− HBED−CC PET/CT imaging to standard choline−based PET/CT. Thirty−seven patients with biochemical relapse of prostate cancer [mean prostate−specific antigen (PSA) 11.1 ± 24.1 ng/ml, range 0.01−116] were retrospectively analyzed after ^{18}F− fluoromethylcholine and ^{68}Ga−PSMA PET/CT

within a time window of 30 days. Radiotracer uptake that was visually considered as prostate cancer was semi− quantitatively analyzed by measuring the maximum standardized uptake values (SUVmax) of the scans acquired 1−hour after injection of ^{68}Ga−PSMA complex solution (median 132 MBq, range 59−263 MBq) and ^{18}F−fluoromethylcholine (median 237 MBq, range 114−374 MBq), respectively. In addition, tumor to background ratios were calculated.

The results showed a total of 78 lesions characteristic for prostate cancer that were detected in 32 patients using ^{68}Ga−PSMA−HBED−CC PET/CT imaging and 56 lesions were detected in 26 patients using choline PET/CT. The higher detection rate in ^{68}Ga−PSMA−HBED−CC PET/CT imaging was statistically significant (p=0.04). In five patients, no lesion was found with both methods. All lesions detected by ^{18}F−fluoromethylcholine PET/CT were also seen by ^{68}Ga−PSMA−HBED−CC PET/CT imaging. In ^{68}Ga−PSMA−HBED−CC PET/CT imaging SUVmax was clearly (>10 %) higher in 62 of 78 lesions (79.1 %) and the tumor to background ratio was clearly (>10 %) higher in 74 of 78 lesions (94.9 %) when compared to ^{18}F−fluoromethylcholine PET/CT.

The authors concluded that ^{68}Ga−PSMA−HBED−CC PET/CT can detect lesions characteristic for prostate cancer with improved contrast when compared to standard ^{18}F−fluoromethylcholine PET/CT, especially at low PSA levels.

iv. **Comparison of PET/CT and PET/MRI hybrid systems using a ^{68}Ga−labelled PSMA ligand for the diagnosis of recurrent prostate cancer: initial experience** (71):

In a more recent publication, the Heidelberg group evaluated the feasibility of PET/MRI imaging with ^{68}Ga−PSMA−HBED−CC. Twenty patients underwent PET/CT 1−hour after injection of the ^{68}Ga−PSMA−HBED−CC followed by PET/MRI 3−hours after injection. Data from the two investigations were first analyzed separately and then compared with respect to tumor detection rate and radiotracer uptake in various tissues. To evaluate the quantification accuracy of the PET/MRI system, differences in SUVs between PET/CT and corresponding PET/MRI were compared with differences in SUVs between PET/CT 1−hour and 3−hours after injection in another patient cohort. This cohort was investigated using the same PET/CT system. With PET/MRI, different diagnostic sequences, higher contrast of lesions and higher resolution of MRI enabled a subjectively easier evaluation of the images. In addition, four unclear findings on PET/CT could be clarified as characteristic of prostate cancer metastases by PET/MRI. However, in PET images of the PET/MRI, a reduced signal was observed at the level of the kidneys (in 11 patients) and around the urinary bladder (in 15 patients). This led to reduced SUVs in six lesions.

SUVmean values provided by the PET/MRI system were different in muscles, blood pool, liver and spleen.

The authors concluded that prostate cancer was detected more easily and more accurately with ^{68}Ga−PSMA PET/MRI than with PET/CT and with lower radiation exposure. Consequently, this new technique could clarify unclear findings on PET/CT. However, scatter correction was challenging when the specific ^{68}Ga−PSMA−HBED−CC was used. Moreover, direct comparison of SUVs from PET/CT and PET/MR needs to be conducted carefully.

These encouraging results suggest ^{68}Ga−PSMA−HBED−CC to be an effective probe for the PC marker "PSMA" in metastatic prostate cancer patients and that it can provide a robust imaging scan to better detect PC tumor burden. We plan to utilize this probe to better understand its diagnostic role in prostate cancer patients with localized disease.

2.6 Investigational Agent

- **^{225}Ac−J591 (IND # 135267)**

The humanized monoclonal antibody−huJ591 is provided by Dr. Neil Bander. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine. ^{225}Ac nitrate residue, 37 MBq (1.0 mCi) is supplied in a 2mL glass vial (by Department of Energy, Oak Ridge Laboratory), a radiochemical grade preparation. **^{225}**Ac−DOTA−huJ591 mAb injection is manufactured by reacting ^{225}Ac chloride with DOTA−huJ591 (3.0 mg) aseptically withdrawn from the packaged drug substance vial and allowing the DOTA chelator to chelate ^{225}Ac in tetramethylammonium acetate buffer (TMAA). Following the reaction, the ^{225}Ac−DOTA−J591 is challenged with an excess of the chelator DTPA to remove any free or loosely bound Ac−225. ^{225}Ac−DOTA−huJ591 is then separated from ^{225}Ac−DTPA by gel filtration (Biogel P−6 column, Biorad, CA) using sterile saline solution containing 2% Human Serum Albumin as an eluent. The eluent fraction (4−8 mL) containing ^{225}Ac−DOTA−huJ591 is then sterilized by membrane filtration into a final drug product vial. QC Samples are removed for Quality Control. The specific activity of the ^{225}Ac−DOTA−huJ591 injection is estimated based on dose calibrator measurement of total ^{225}Ac activity (50 – 300μCi) and the total DOTA−huJ591 (3 mg) precursor used. Expected SA is 16.6 – 100 μCi/mg. Labeling efficiency and radiochemical purity will be determined using ITLC. Immunoreactivity of radiolabeled J591 mAb preparations will be determined using PSMA+ LNCaP tumor cells.

The material produced are subjected to quality assurance testing as outlined in the Food and Drug Administration "Points to Consider". All production data and supporting documents, together with the results of all quality assurance testing, have been provided to and reviewed by the Food and Drug Administration (^{225}Ac−J591, IND# **135267**).

- **^{68}Ga−PSMA−HBED−CC (IND # 124495)**

PSMA has recently been discovered as a promising target for radioisotope based approaches for PET imaging, for example, using ^{68}Ga−labeled PSMA ligands(61-66). All of these trials report retrospective data on case reports/case series. No well−designed prospective clinical trial has been published/reported yet.

During the last two decades, many efforts have been undertaken to develop PSMA−ligands(67, 68). One of these ligands, the small molecule Glu−NH−CO−NH−Lys−(Ahx)−[^{68}Ga(HBED−CC)], also known as PSMA−11®, PSMAHBED, Glu−CO−Lys(Ahx)−HBED−CC, DKFZ−PSMA−11, PSMA−HBED− CC, PSMA−HBED, PSMA or Prostamedix™, developed at the German Cancer Research Center Heidelberg (DKFZ), has become the most clinically used radiotracer. This compound shows a strong binding affinity to PSMA as well as a highly efficient internalization into PCa cells(69, 70). PET/CT−imaging with ^{68}Ga−PSMA−11 has demonstrated this novel method as an important imaging modality for diagnosing recurrent PCa(61, 71-73). Perera et al., did systematic review of ^{68}Ga−PET articles and Sixteen articles involving 1309 patients were analyzed. On per−patient analysis, the summary sensitivity and specificity were both 86%. On per lesion analysis, the summary sensitivity and specificity were 80% and 97%, respectively(74).

[redacted] produces PSMA−HBED−CC for clinical use. Upon purchase and shipment to Weill Cornell Medicine, the peptide will be labeled with ^{68}Ga with the final product being ^{68}Ga−PSMA−HBED−CC. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine.

The material produced are subjected to quality assurance testing as outlined in the Food and Drug Administration “Points to Consider”. All production data and supporting documents, together with the results of all quality assurance testing, have been provided to and reviewed by the Food and Drug Administration (^{68}Ga−PSMA−HBED−CC, IND# 124495).

2.7 Rationale

Prostate cancer (PC) is the most common solid male cancer. For those patients with clinically organ−confined disease, radical prostatectomy (RP) remains the most commonly used treatment modality (76). However, even after surgical treatment, high risk PC is prone to recur and often becomes resistant to conventional hormonal therapy (77). For this reason, efforts have been made to optimize patient outcome in the adjuvant and neoadjuvant settings (78-80). Thus far, neoadjuvant therapies have yet to demonstrate a survival benefit (81). Therefore, newer strategies to prevent or rescue PC recurrences are highly desirable.

It is now recognized that evasion of an effective immune response is a hallmark of cancer (82). Immunotherapy may induce tumor cell death with a relatively high degree of specificity unlike hormonal or chemotherapy, which has a non−specific action. PC is an ideal target for immunotherapy because of its indolent nature which allows adequate time to activate the immune system (83), and in fact, the first therapeutic cancer vaccine to be approved was for PC (10). Identification of PC tumor associated antigens (TAA) has provided ideal targets for

immunotherapy, with prostate−specific membrane antigen (PSMA) as one of the most relevant targets in oncology as a highly restricted cell−surface membrane protein that is not secreted (18, 19, 84, 85).

Based on the knowledge that certain tumor types express significant amounts of one or several specific cell−surface proteins, the development of drugs aimed at these targets is becoming an increasingly important component in the treatment of cancer. One example is the successful use of the mAb rituximab in patients with non−Hodgkin's lymphoma (NHL). Rituximab targets CD20 which is expressed on the surface of 95% of B cells in NHL (86). Another clinically validated target is human epidermal growth factor receptor 2 (HER2), which in breast cancer is targeted by trastuzumab (87). In PC, several studies, which will be addressed throughout this protocol, have emphasized PSMA as both a diagnostic and therapeutic target. Similar to prostate−specific antigen (PSA), PSMA is a highly expressed, prostate−specific biomarker. While PSMA is not a novel discovery, it remains a highly relevant target with the knowledge that attenuated AR signaling even prior to hormonal therapy is the hallmark of lethal PC and recent studies have confirmed the reciprocal relationship between PSMA and AR signaling in the era of highly potent AR−directed therapy(26, 88-92).

2.8 Rationale for the appropriate radioisotope:

Radionuclides that emit alpha particles (helium nucleus consisting of 2 protons and 2 neutrons) deliver about 4,000−fold the energy of a beta particle (composed of an electron or positron). While thousands of beta particle hits are required to kill a cell, a single alpha hit is sufficient to kill by causing irreparable double strand DNA breaks. In addition to their high energy, the range of emission is only 50−100μ leading to less potential for toxicity to bystander cells. Based upon strong early studies, an international phase 3 study led by co−investigator Dr. Sartor demonstrated an overall survival benefit in addition to an improvement in patient reported outcomes, leading to FDA approval (13, 43). Importantly, as predicted based upon the short path length, the toxicity profile of ^{223}Ra (Radium−223) is favorable with only mild myelosuppression and gastrointestinal adverse events. Very clearly, this established α−emitting radiopharmaceutical in the clinic. However, ^{223}Ra is "targeted" simply because it chemically resembles calcium and is, therefore, preferentially deposited in sites of active bone formation. Because the bone−targeting radiopharmaceutical anti−tumor effect derives from accumulation in proximity to malignant cells and/or stroma, these agents entirely ignore soft tissue and extra−osseous visceral metastases. As such, a method of delivering an α−emitting particle directly to tumor cells would be a significant advance.

Converting indirect bone targeting to direct tumor cell targeting via a PSMA−specific mAb will result in the alpha particle being internalized and deposited adjacent to the nucleus. Such proximity to the nucleus (and the DNA) yields even greater potency. In addition, unlike the ^{223}Ra indirect approach, direct tumor targeting would enable treatment of PC outside the bone (e.g., in the soft tissue and circulation) and allow killing of bone marrow micro−metastases before they induce the increased bone turnover necessary for successful use of ^{223}Ra. We expect that by targeting macro− and microscopic metastatic tumor sites with PSMA−targeted alpha particles, we will be able to

demonstrate even greater efficacy than seen with β−emitters, potentially with less toxicity. There have been anecdotal reports of PSMA−targeted α−particles utilizing actinium−225 (^{225}Ac) in Europe, including a recent publication with impressive results(44). In addition, there are unpublished reports of "success" with PSA declines and scan improvements, with issues of impaired quality of life due to salivary gland toxicity. However, like the experience with PSMA−targeted β−radiation, there are no prospective trials. Based upon the high potency of α−particles, it may be even more important to target tumor rather than the other sites of low−levels of PSMA expression such as proximal renal tubules, salivary glands, and small bowel. In fact, a recent published report demonstrated significant proximal renal tubule uptake and late radiation nephropathy in mice models(45). Therefore, mAb−based α−particle delivery should be advantageous with less expected myelosuppression than seen with β−emitters.

2.9 Risk/Benefit Assessment

This is a phase I clinical trial with the primary endpoints to find DLT and MTD. Based on pre−clinical findings by our group combined with our prior human experience with ^{90}Y−J591 and ^{177}Lu−J591 the dose−limiting organs/tissue is expected to be red marrow. The expected toxicities can be leukopenia, thrombocytopenia, anemia, fatigue, renal failure, and transaminitis.

2.10 Correlative Studies Background

i. Archival tissue and cell−free plasma DNA to assess genomic alterations:

PC is a clinically and molecularly heterogeneous disease with marked variability in patient outcomes. Defining the genomic alterations in PC has improved classification of tumors: for instance, 50% harbor TMPRSS2-ERG gene fusion, 10% SPOP mutation, and germline or somatic alterations involving DNA repair pathways (eg., BRCA2, ATM, MMR defects) occur in 20% of CRPC. Based on recent data from our group and others, we predict that these genomic alterations may help identify patients most likely to respond to DNA damaging therapy with ^{225}Ac-J591. In PC models, ERG expression results in relative resistance to ionizing radiation. Conversely, SPOP mutant prostate cancers and/or those with CHD1 loss show increased genomic instability, play a role in double strand DNA break repair, and result in increased sensitivity to DNA damage. Germline or somatic alterations involving DNA damage repair genes (such as BRCA2 and ATM) have also been shown to be preferentially sensitive to DNA damaging therapy including PARP inhibitors and platinum chemotherapy. We therefore hypothesize that distinct molecular phenotypes of prostate cancer may have predictable and exploitable differences in sensitivity to DNA damaging agents such as actinium-225. We and others have also described prostate cancer subtypes based upon AR signaling and neuroendocrine differentiation. We hypothesize that the NEPC / AR low tumors would express low PSMA and respond less well to PSMA-targeted therapy. We will test these hypotheses using tissue and ctDNA collected from our prospective cohorts.

We will evaluate archival tumor tissue (preferentially from metastatic biopsies) with the plan for utilization of our CLIA-approved targeted platform covering DNA (for mutation and copy number) and RNA (for fusion analysis) of 150 cancer related genes including AR, SPOP, CHD1, DNA repair, ERG fusion, and other CRPC-relevant alterations. Gene expression of androgen signaling genes and other relevant pathways will be assessed using a custom panel Nanostring platform developed at WCM, involving 350 genes including the AR signaling signature genes (n=30), the AR V7 splice variant, neuroendocrine prostate cancer genes, epithelial mesenchymal transition, cell cycle- genes, TMPRSS2-ERG fusion transcript, and control/housekeeper genes. In addition, we will collect plasma samples for ctDNA analysis prior to treatment, at 3-months, and at progression using the prostate cancer specific PCF SELECT platform developed in collaboration with others as part of a PCF Challenge Award. As discussed above, we will describe findings from this exploratory endpoint in each study with the plan to analyze these findings with relationship to efficacy and toxicity across studies, in particular at doses found to have efficacy.

ii. **CTC Count:**

Circulating tumor cell (CTC) counts via the CellSearch platform were demonstrated to be prognostic in men with advanced prostate cancer prior to systemic therapy and a "conversion" from an unfavorable count (≥5 CTCs/7.5 mL) was associated with a median similar to those starting with favorable counts, leading to clearance of this particular test by the FDA (93). More recently, the combination of CellSearch CTC enumeration and serum LDH have been demonstrated to have prognostic value, meeting Prentice criteria for survival surrogacy in the setting of abiraterone/prednisone treatment in men with mCRPC previously treated with docetaxel (94) and in a large analysis of 5 phase III trials, conversion from unfavorable to favorable as well as undetectable CTC count at 12 weeks is strongly associated with overall survival.

iii. **Immune correlates**:

We and others have observed some durable responses following radiolabeled-PSMA therapy. Radiation may lead to an induction of or increase in immune responses to locally expressed antigens, but unlike external beam radiation, PSMA-targeting generally delivers radiation to all sites of disease (by imaging) and therefore observing an abscopal response is not possible. We plan to assess this by analyzing serological changes pre- and post-treatment and will also explore induction of immunogenic cell death by assessment of HMGB1 and calreticulin.

iv. **Molecular imaging:**

While there is an increasingly large body of clinical data for PSMA PET imaging (above), reproducibility of imaging has not been well studied in a test – retest format.

3. SUBJECT SELECTION

3.1 Study Population

Subjects who have documented progressive metastatic CRPC disease, who meet the inclusion and exclusion criteria will be eligible for participation in this study.

3.2 Inclusion Criteria

1. Histologically or cytologically confirmed adenocarcinoma of prostate

2. Documented progressive metastatic CRPC based on Prostate Cancer Working Group 3 (PCWG3) criteria, which includes at least one of the following criteria:

    i. PSA progression
    ii. Objective radiographic progression in soft tissue
    iii. New bone lesions

3. ECOG performance status of 0−2

4. Have serum testosterone ≤ 50 ng/dL. Subjects must continue primary androgen deprivation with an LHRH analogue (agonist/antagonist) if they have not undergone bilateral orchiectomy.

5. Have previously been treated with at least one of the following:

    - Androgen receptor signaling inhibitor (such as enzalutamide)

    - CYP 17 inhibitor (such as abiraterone acetate)

6. Have previously received taxane chemotherapy, been determined to be ineligible for taxane chemotherapy by their physician, or refused taxane chemotherapy.

7. Age ≥ 18 years

8. Patients must have normal organ and marrow function as defined below:

    Absolute neutrophil count ≥2,000 cells/mm^3
    Hemoglobin ≥9 g/dL
    Platelet count ≥150,000 x $10^9/\mu L$
    Serum creatinine ≤1.5 x upper limit of normal (ULN) or calculated creatinine clearance ≥ 60 mL/min/1.73 m^2 by Cockcroft−Gault
    Serum total bilirubin ≤1.5 x ULN (unless due to Gilbert's syndrome in which case direct bilirubin must be normal
    Serum AST and ALT ≤3 x ULN in absence of liver metastases; <5x ULN if

due to liver metastases (in both circumstances, bilirubin must meet entry criteria)

9. Ability to understand and the willingness to sign a written informed consent document.

3.3 Exclusion Criteria

1. Implantation of investigational medical device ≤4 weeks of Cycle 1, Day 1 or current enrollment in oncologic investigational drug or device study

2. Use of investigational drugs ≤4 weeks or <5 half−lives of Cycle 1, Day 1 or current enrollment in investigational oncology drug or device study

3. Prior systemic beta−emitting bone−seeking radioisotopes (e.g. Sm-153, Sr-89)

4. Known active brain metastases or leptomeningeal disease

5. History of deep vein thrombosis and/or pulmonary embolus within 1 month of C1D1

6. Other serious illness(es) involving the cardiac, respiratory, CNS, renal, hepatic or hematological organ systems which might preclude completion of this study or interfere with determination of causality of any adverse effects experienced in this study

7. Radiation therapy for treatment of PC ≤4 weeks of Day 1 Cycle 1

8. Patients on stable dose of bisphosphonates or denosumab, which have been started no less than 4 weeks prior to treatment start, may continue on this medication, however patients are not allowed to initiate bisphosphonate/denosumab therapy during the DLT−assessment period of the study.

9. Having partners of childbearing potential and not willing to use a method of birth control deemed acceptable by the principle investigator and chairperson during the study and for 1 month after last study drug administration

10. Currently active other malignancy other than non−melanoma skin cancer. Patients are considered not to have “currently active” malignancy if they have completed any necessary therapy and are considered by their physician to be at less than 30% risk of relapse.

11. Known history of known myelodysplastic syndrome

4. OVERVIEW OF STUDY DESIGN AND METHODOLOGY

4.1 Study Design

This is an open−label, single−center Phase I dose escalation study designed to determine the dose−limiting toxicity (DLT) and the maximum tolerated dose (MTD) of ^{225}Ac−J591 in a single dose regimen. The dose escalation will start at 13.3 KBq/Kg (0.36 μCi/Kg) and escalate in increments of 13.3 KBq/Kg (0.36 μCi/Kg) for each dose to a maximum of 93.3 KBq/Kg (2.52 μCi/Kg).

Patients must have documented progressive metastatic CRPC disease based on Prostate Cancer Working Group 3 (PCWG3) criteria in order to be eligible for enrollment. Upon meeting the inclusion and exclusion criteria and signing the informed consent and HIPPA form, subjects will undergo the screening. As part of the screening, subjects will get a single dose of ^{68}Ga−PSMA−HBED−CC and will have a PET/CT. Nuclear Medicine physician(s) will review the PET/CT scans to document PSMA expression at tumor site(s).

A single dose of ^{225}Ac−J591 will be given to subjects with documented progressive metastatic CRPC. The ^{225}Ac dose (13.3 KBq/Kg – 93.3 KBq/Kg or 0.36 μCi/Kg – 2.52 μCi/Kg) will be escalated in up to 7 different dose levels (utilizing an accelerated dose−escalation study design). Initially single subject cohorts will be enrolled in Cohort 1 (13.3 KBq/Kg or 0.36 μCi/Kg). Subjects will be closely monitored for AEs (weekly x2 weeks, then every 2 weeks for one month, at 8 and 12 weeks, then every 4 weeks for next 3 months and then every 6 months for upto 3 years as described in Table :4). If a subject at a specific dose−level does not experience any grade >1 attributable toxicity (or increase by more than 1 grade for pre-existing AE), the next subject will be enrolled in the next cohort. Once any subject at any dose level experiences attributable Gr >1 toxicity OR enrollment begins at dose-level 5, transition to modified 3+3 dose−escalation study design will occur. Any grade ≥3 non-hematological toxicity or any grade 4 hematological toxicity with additional complications (grade 4 neutropenia [ANC <500/mm^3] lasting greater than 7 days or of any duration associated with fever (i.e. febrile neutropenia); severe grade 4 thrombocytopenia (platelet count < 15,000) lasting greater than 7 days, associated with hemorrhage, and/or requirement of > 3 platelet transfusions in a 30-day period) at the a specific ^{225}Ac−J591 dose will determine DLT and MTD. The enrollment ceiling of the dose escalation portion of the study is up to 52 study participants (up to 7 groups of up to 6 subjects each + 10 subjects in the expansion cohort).

Upon completion of investigational treatment with single dose of ^{225}Ac−J591, subjects will undergo ^{68}Ga−PSMA−HBED−CC injection and same day PET/CT at the end of study visit to document treatment response. Subsequently survival data and additional treatment(s) information will be captured from their routine Standard of care (SOC) visits.

Table 3: Treatment Plan with ^{225}Ac−J591

Cohort	Treatment Dose		n
	KBq/Kg	μCi/Kg	
1	13.3	0.36	1−6
2	26.7	0.72	1−6
3	40.0	1.08	1−6
4	53.3	1.44	1−6
5	66.7	1.80	3−6
6	80.0	2.16	3−6
7	93.3	2.52	3−6

4.2 Rationale for Dose−Escalation Strategy:

This study will use an accelerated dose−escalation design, transitioning to a 3+3 dose−escalation study design upon the occurrence of grade > 1 attributable toxicity or reaching Cohort 5, with the planned initial and subsequent cohorts described in **Table 3**. Briefly, this design is constructed to reduce the chance of escalating the dose when the probability of DLT is high, and increase the chance of escalating the dose when the probability of DLT is low with details provided in Section 12.1.

5. REGISTRATION PROCEDURES

5.1 Identification of subjects:

Patients diagnosed with documented progressive metastatic CRPC disease who are visiting Oncology Clinic at NYPH−Cornell Campus for their standard of care visit, will be approached for recruitment for this study. Investigators or delegates under their direct supervision may perform pre−screening of these potential subjects.

5.2 Consent process

Potential subjects will have a discussion with the investigator/delegate including the rationale for the study, investigational nature of the protocol and study drug and the voluntary nature of participation, potential risks and benefits, alternatives to participation, and study procedures. Individuals will have the opportunity to read the written informed consent document at their leisure (preferably outside of the clinical area for > 1 day) and the opportunity to have questions answered in a private location with the understanding that should they decide not to participate, they will still be able to receive any available standard of care therapy. Potential subjects will also have the opportunity to obtain the advice of their treating physician. Investigators or delegates under their direct supervision will verify the subject's understanding of the investigational and voluntary nature of the study, the potential risks and benefits, study procedures, and alternatives prior to signing of the written informed consent**.**

5.3 Central Patient Registration

Subjects will be assigned a sequence number for the protocol and will be centrally registered with the Weill Cornell Medicine (WCM), Division of Hematology and Medical Oncology Clinical Research Office with the following documents:

a. WCM Patient registration form
b. First and last page of the fully executed informed consent form (including HIPPA), plus additional pages if checkboxes for correlative studies are required.
c. Eligibility checklist signed and dated by investigator and research nurse
d. Documentation of any eligibility waivers granted
e. Entry of screening information into WCM web–based system

Central registration information is reviewed and entered into the HemOnc centralized research database. Documentation of patient registration will be confirmed prior to release of study agent by nuclear medicine.

6. STUDY PROCEDURES

Screening assessments and study procedures outlined in this section can only be performed after obtaining informed consent. All on–study visits and dosing should be scheduled from Day 1 (date of the first infusion) on the study. It is very important that protocol procedures are performed at the time–points stipulated below. When it is not possible to perform, the study visit at the exact time–point, the visit maybe performed within the acceptable visit window as defined in the visit–specific section below.

After obtaining informed consent from enrolled subject(s), screening and study related treatment procedures will be performed as outlined in Table 4 and described in detail in Section 6.1.

6.1 Schedule of Evaluations

Table 4: Schedule of trial events

	Screening	Treatment Visit	F/U Visit 1	F/U Visit 2	F/U Visit 3	F/U Visit 4	F/U Visit 5	F/U Visit 6	Scan / Efficacy Visit	Short Term F/U[j]	Long Term F/U
Informed Consent	x										
Demographics	x										
Medical History	x	x	x	x		x		x	x	x	
Physical Exam	x	x	x	x		x		x	x	x	
Performance Status	x	x				x		x	x	x	
Vital Signs[a]	x	x	x	x		x		x	x	x	
PRO[b]	x	x	x			x		x	x		
CBC with diff, plts[c]	x	x	x	x	x	x	x	x	x	x	x[k]
Serum Chemistry[d]	x	x	x	x	x	x	x	x	x	x	x[k]
PSA	x	x		x		x		x	x	x	
LDH		x		x		x		x	x	x	
Testosterone	x								x		
CTC Count[e]	x	x[e]							x		
Cell−Free DNA Research sample[f]	x	x[f]							x	x[f]	
Seromics Research sample[f]	x	x[f]							x		
CTC Research sample	x	x[f]							x		
^{68}Ga−PSMA infusion & PET CT[g]	x[g]								x		
Radiographic evaluation[h]	x								x	x[h]	x[h]
^{225}Ac−J591 infusion		x									
Archival Tissue	x										
Stool	x	x[f]									
Adverse Event Monitoring	x	x	x			x		x	x	x	x[k]
Concurrent Medications	x	x	x			x		x	x	x	
Survival Assessment[i]											x

a: Vital signs will include height and weight during screening and at least weight thereafter
b: PRO = patient reported outcomes = BPI−SF and FACT−P
c: Any subject with grade 4 neutropenia or thrombocytopenia at any time point will be required to have CBC with differential at least twice per week
d: CMP (with direct bilirubin in subjects with known Gilbert's syndrome)
e: CTC enumeration via CellSearch methodology may be obtained during screening or prior to treatment C1D1
f: Blood/stool samples for research may be obtained during screening or prior to treatment C1D1, within 2 weeks of scan/efficacy visit, and progression
g: PSMA imaging is planned for all subjects. However, in the event of unavailability of radiotracers, subjects will be allowed to enroll and receive treatment without PSMA imaging. Optional repeat ^{68}Ga-PSMA PET/CT will be repeated prior 6-48 hours later to treatment in consenting subjects.
h: Radiographic evaluation will include bone scan, CT/MRI of abdomen/pelvis, and Chest x−ray (CXR waived if CT/MRI includes chest). Following mandatory repeat imaging with Scan/Efficacy visit, continued repeat imaging is recommended approximately 12 weeks until radiographic progression as part of standard care.
i: Survival assessment and relevant medical history to be collected until death
j: Short−term follow up to be completed q4 weeks following the scan visit until 6 months from the 1st treatment visit
k: Long-term follow up to be completed q6 months for up to 3 years following the initial 6-month assessment; should include CBC with differential and CMP (in any CLIA certified lab) and long-term AE assessment (defined as either physician history and physical or phone assessment by member of the study team plus review of any additional medical records if deemed necessary to assess attribution of adverse event).

6.1.1. Screening Visit

The following procedures must be completed no more than 1 month prior to enrollment and no more than 4 weeks following enrollment (except PSMA PET and research samples, which may be performed just prior to treatment if necessary due to scheduling issues).

- Informed Consent
- Demographics
- Medical History
- Previous therapy
- Surgical report will include date and type of surgery +/− lymphadenectomy
- Radiotherapy report will include modality of therapy with prescribed dose and field and dates of therapy
- Previous systemic (hormonal, chemo, other) therapy – drugs, doses, dates of therapy
- Complete Physical Exam including height and weight
- Patient reported outcomes: BPI−SF and FACT−P
- Vital Signs
- ECOG Performance Status
- CBC with differential and platelet count
- CMP (with direct bilirubin with known Gilbert's syndrome)
- PSA
- Testosterone
- CTC count via CellSearch methodology (may be collected prior to treatment visit, do not need to repeat if has known result within 1 month of C1D1)
- Research blood collect blood collection (plasma DNA, serum, CTC) - may be collected any time prior to treatment C1D1
- CT or MRI (abdomen−pelvis), up to 1 month prior to enrollment
- Bone scan, up to 1 month prior to enrollment

- Any confirmatory tests to assess equivocal results of bone scan should also be completed within a month of enrollment
- CXR, up to 1 month prior to enrollment (CXR waived if CT chest performed)
- ^{68}Ga−PSMA−HBED−CC (5±2mCi or 185±74MBq) injection followed by PET/CT
- Optional repeat ^{68}Ga−PSMA−HBED−CC injection and PET/CT 6-48 hours after initial injection/scan in consenting subjects for research purposes
- Archival tissue collection for research
- Stool collection for research – may be collected any time following consent prior to treament on C1D1

Screening (except required ^{68}Ga−PSMA−HBED−CC PET/CT scan) and treatment visit may occur on the same day provided results are available, all entry criteria are met, and the subject is registered on the study prior to dosing. In this instance, duplicate procedures do not need to be performed. The optional 2nd ^{68}Ga−PSMA−HBED−CC PET/CT scan may occur prior to treatment on Day 1.

6.1.1.1 Re–screening

Subjects who are unable to complete the initial screening or are not initially eligible will be permitted to undergo repeat screening (following repeat written informed consent).

6.1.2 Treatment Phase

The treatment and early monitoring phase comprises of **7** visits spanning over approximately 8−weeks. Based upon 3 prior studies of single-dose radiolabeled J591 with nearly all toxicity occurring from 3-5 weeks following dosing (and blood count nadirs at days 28-31), subjects will be monitored most closely (at least weekly) during this time.

Neutrophil count, platelet count, bilirubin, transaminases, and serum creatinine must be performed with results available and within range of eligibility criteria within 1 week prior to treatment visit.

Details for each visit are listed below:

6.1.2.1 ^{225}Ac−J591 infusion (Treatment Visit, Day 1)

The following procedures must be completed on the day of treatment with ^{225}Ac−J591:

- Targeted Physical Examination with vital signs and weight
- Medical History
- Brief Pain Inventory, FACT−P
- ECOG Performance Status
- CBC with differential and platelet count

- CMP (with direct bilirubin with known Gilbert's syndrome)
- PSA
- LDH
- Research blood collection (plasma DNA, serum, CTC) – if not done during screening
- Single intravenous dose of ^{225}Ac−J591 with at least 1 liter normal saline (Dose based on the Cohort in which the subject is enrolled)
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures

As stated above, blood counts, bilirubin, transaminases, and renal function assessment must be available within 1 week prior to treatment visit and meet eligibility criteria.

6.1.2.2 Follow−up visits: Follow up Visit 1 (Day 8 ±1), Visit 2 (Day 15 +/-2), Follow up Visit 4 (Day 29 ±2), Follow up Visit 6 (Day 57 ±2)

- Targeted Physical Examination with vital signs and weight
- Medical History
- Brief Pain Inventory, FACT−P (except Follow Up Visit 2)
- ECOG Performance Status (except Follow Up Visit 2)
- CBC with differential and platelet count
- CMP (with direct bilirubin with known Gilbert's syndrome)
- PSA (Follow up visit 2 and 4)
- LDH (Follow up visit 2 and 4)
- Adverse event evaluation
- Concomitant medications

6.1.2.3 Follow−up lab visits: Follow up Visit 3 (Day 22 ±2), Visit 5 (Day 43 ±3)

- CBC with differential and platelet count
- CMP (with direct bilirubin with known Gilbert's syndrome)

Lab visits 3 and 5 may be performed at any CLIA certified lab

In addition, should there be any period of <u>grade 4 neutropenia or thrombocytopenia</u>, then <u>CBC with differential is required at least twice per week</u> (defined as a 7-day period)

6.1.3 Efficacy Evaluation (scan) Visit (Day 85 ± 7)

The following procedures must be completed during the end of study visit (likely occurring on at least 2 separate days):

- Targeted Physical Examination with vital signs and weight
- Medical History
- Brief Pain Inventory, FACT−P
- ECOG Performance Status
- CBC with differential and platelet count
- CMP (with direct bilirubin with known Gilbert's syndrome)
- PSA
- LDH
- Testosterone
- CTC count via CellSearch methodology
- Research blood collection (plasma DNA, serum, CTC)
- Same standard radiographic imaging modality as baseline (CT/MRI and bone scan +/− CXR)
- Single intravenous dose of ^{68}Ga−PSMA−HBED−CC (5±2mCi or 185±74MBq) followed by PET/CT
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures

6.1.4 Short term follow up (q4 week visits x3 following Efficacy/Scan visit)

Unless subjects withdraw or initiate new treatment, the following procedures must be completed every 4 weeks (+/− 7 days) after the scan visit until radiographic progression occurs:

- Targeted Physical Examination with vital signs and weight
- Interim Medical History
- ECOG Performance Status
- CBC with differential and platelet count
- CMP (with direct bilirubin with known Gilbert's syndrome)
- PSA
- LDH
- At progression: Above procedures plus plasma DNA collection for research

These visits may be performed with any licensed provider at any CLIA certified lab, provided that the results are available/provided to the Investigator within allowable windows. Repeat CT and bone scan are recommended with the final short−term follow up visit (which will occur after radiographic progression per PCWG3 recommendations).

6.1.5 Long Term Follow Up

Long-term follow up to be completed q6 months for up to 3 years following the initial 6-month assessment (may be included during short term follow up); should include CBC with differential and CMP (in any CLIA certified lab) and long-term AE assessment (defined as either physician history and physical or phone assessment by member of the study team plus review of any additional medical records if deemed necessary to assess attribution of adverse event). Survival assessment and relevant medical history to be collected until death. Information may be collected from external providers. Should a subject withdraw from short-term follow up per protocol (i.e. completing study procedures including imaging and visits to the WCM site every 12 weeks until radiographic progression), per PCWG3 we recommend follow up CT and bone scans every 12 weeks per clinical standard of care until radiographic progression.

6.2 Treatment Administration

Treatment will be administered on an *outpatient* basis. Reported adverse events and potential risks are described in **Section 13**.

6.2.1 Agent Administration

Treatment will be administered only to eligible subjects under the supervision of the investigator or identified co−investigator(s). Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in **Section 13**. Appropriate dose modifications/delays of the study drug are described in **Section 7**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's malignancy.

6.2.2. Study drug preparation

A. ^{225}Ac−J591

The humanized monoclonal antibody−huJ591 is provided by Dr. Neil Bander. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine. ^{225}Ac nitrate residue, 37 MBq (1.0 mCi) is supplied in a 2mL glass vial (by Department of Energy, Oak Ridge Laboratory), a radiochemical grade preparation. ^{225}Ac−DOTA−huJ591 mAb injection is manufactured by reacting ^{225}Ac chloride with DOTA−huJ591 (3.0 mg) aseptically withdrawn from the packaged drug substance vial and allowing the DOTA chelator to chelate ^{225}Ac in tetramethylammonium acetate buffer (TMAA).

Following the reaction, the ^{225}Ac−DOTA−J591 is challenged with an excess of the chelator DTPA to remove any free or loosely bound Ac−225. ^{225}Ac−DOTA−huJ591 is then separated from ^{225}Ac−DTPA by gel filtration (Biogel P−6 column, Biorad, CA) using sterile saline solution containing 2% Human Serum Albumin as an eluent. The eluent fraction (4−8 mL) containing ^{225}Ac−DOTA−huJ591 is then sterilized by membrane filtration into a final drug product vial. QC Samples are removed for Quality Control. The specific activity of the ^{225}Ac−DOTA−huJ591 injection is estimated based on dose calibrator measurement of total ^{225}Ac activity (50 – 300μCi) and the total DOTA−huJ591 (3 mg) precursor used. Expected SA is 16.6 – 100 μCi/mg. Labeling efficiency and radiochemical purity will be determined using ITLC. Immunoreactivity of radiolabeled J591 mAb preparations will be determined using PSMA+ LNCaP tumor cells.

The material produced are subjected to quality assurance testing as outlined in the Food and Drug Administration "Points to Consider". All production data and supporting documents, together with the results of all quality assurance testing, have been provided to and reviewed by the Food and Drug Administration (^{225}Ac−J591, IND# **135267**).

B. ^{68}Ga−PSMA−HBED−CC

[redacted] produces PSMA−HBED−CC. Upon purchase and shipment to Weill Cornell Medicine, they will be labeled with ^{68}Ga with final product being ^{68}Ga−PSMA−HBED−CC. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine.

The material produced are subjected to quality assurance testing as outlined in the Food and Drug Administration "Points to Consider". All production data and supporting documents, together with the results of all quality assurance testing, have been provided to and reviewed by the Food and Drug Administration (^{68}Ga−PSMA−HBED−CC, IND# 124495).

6.2.3 Route of Administration

^{225}Ac−J591 and ^{68}Ga−PSMA−HBED−CC will be administered intravenously.

6.2.4 Dose levels for ^{225}Ac−J591

Subject(s) enrollment will be done in an accelerated dose−escalation study design at each dose level (**13.3 KBq/Kg or 0.36 μCi/Kg**). The enrollment ceiling of the dose escalation portion of the study is up to 42 study participants (up to seven groups, up to 6 at each dose level). Each patient would receive single dose of the investigational agent. The total dose received by a subject will vary from **13.3 KBq/Kg to 93.3 KBq/Kg or (0.36 μCi/Kg – 2.52 μCi/Kg)**.

6.2.5 Dose levels for ^{68}Ga−PSMA−HBED−CC

All subjected enrolled will receive 5 ±2mCi or 185 ±74MBq of ^{68}Ga−PSMA−HBED−CC during the screening visit and scan/efficacy visit.

6.2.6 Study drug premedication

No pre−medication is required, but recommended pre−medication for ^{225}Ac−J591 will be H1 blocker (recommended 25−50 mg diphenhydramine) and acetaminophen (recommended 325−620 mg). No pre−medications will be administered for ^{68}Ga−PSMA−HBED−CC.

6.2.7 Study drug administration

Intravenous access (peripheral or central) must be well established prior to initiating infusion. At the time of dosing, the IV line will be connected to an infusion container containing the prepared volume of ^{225}Ac−J591 or ^{68}Ga−PSMA−HBED−CC. ^{225}Ac−J591 will be infused at a rate of no faster than 5 mg/minute. ^{68}Ga−PSMA−HBED−CC may be administered as slow IV push. In addition, a minimum of 1 liter of normal saline will be administered prior to or following ^{225}Ac−J591 infusion and subjects should be instructed to increase oral hydration for the week following treatment.

6.2.8. Monitoring Vital signs pre/post ^{225}Ac−J591 and ^{68}Ga−PSMA−HBED−CC

The infusion of ^{225}Ac−J591 and subsequent monitoring will occur in a facility that is equipped for cardio–pulmonary resuscitation. The dispensed dose will be infused under the supervision of nuclear medicine physician or designee under the supervision of a nuclear medicine physician. Infusion–related reactions (fever, rigors) will be treated with acetaminophen, meperidine and diphenhydramine hydrochloride as clinically appropriate. Other allergic events will be managed as follows: rash, pruritus, urticaria and wheezing will be treated with diphenhydramine hydrochloride, meperidine and/or steroids as clinically appropriate. Anaphylaxis or anaphylactoid signs or symptoms will be treated with steroids and/or epinephrine as clinically indicated. Vital signs will be monitored during the infusion. Systolic and diastolic blood pressure (mm Hg), temperature, pulse rate (beats/minute), and respiratory rate (breaths/minute), will be recorded with the patient in sitting position. Any clinically significant change in the vital signs will be recorded as AEs.
Serial vital signs including temperature, BP, and heart rate will be monitored within 30 minutes before the infusion, within 30 minutes and at 60 minutes (±10 minutes), at 90 ±10 minutes, and 120 ±10 minutes following start of ^{225}Ac−J591 infusion. If any subject has any infusion-related adverse reaction within 120 minutes for ^{225}Ac−J591, they will stay longer until it is resolved.

6.2.9. Imaging Plan

^{68}Ga−PSMA−HBED−CC PET/CT Scan

Patient preparation should be according to the policies and procedures of the local imaging site (CBIC). The patient does not need to be fasting for either the infusion or the scans. The use of intravenous or oral contrast will not be permitted. Specifications for acquiring the ^{68}Ga−PSMA−HBED−CC PET/CT scans will be provided in study specific documentation by the study chair or the co−investigators from the Division of Nuclear Medicine. PET/CT should be obtained during the screening visit as well as at the end of study visit. The images are acquired between 1 and 3 hours after the ^{68}Ga−PSMA−HBED−CC infusion. Image acquisition will be from vertex of skull to mid thighs.

6.2.10 Managing toxicity

NCI CTCAE version 4.0 is used to grade all adverse events.

Dose−limiting toxicity (DLT) for both single-subject and 3+3 dose-escalation cohorts is defined as:

- Grade 4 neutropenia lasting longer than 1 week or any occurrence of febrile neutropenia
- Grade 4 thrombocytopenia lasting longer than 1 week, requiring more than 2 platelet transfusions in a 30-day period, or resulting in major bleeding
- Any grade > 2 non−hematologic toxicity deemed to be at least possibly related to ^{225}Ac−J591 will be termed as dose−limiting toxicity.

Maximum Tolerated Dose (MTD) is defined as:

- The dose that produces an "acceptable" level of toxicity or that, if exceeded, would put subjects at "unacceptable" risk for toxicity. Definition of the MTD usually relies on the sample, as MTD is defined as the dose level at which no more than two patients out of six experienced dose−limiting toxicity (DLT).

Note: Toxicities as described above will be considered DLT if they are at least possibly related to ^{68}Ga−PSMA−HBED−CC or ^{225}Ac−J591 as judged by the investigator. Attribution will be reviewed by the study chair and discussed with the medical monitor if there are questions about severity or attribution.

6.3 General Concomitant Medication and Supportive Care Guidelines

All medications that are administered during the study must be recorded in the patient's CRF and in the source documents. Concomitant medications for other medical conditions are permitted as clinically indicated subject to approval by the study chair.

Subjects will be advised to use contraceptive precautions to avoid pregnancy during the treatment phase since the effects of investigational agents on sperms and embryos are unknown.

6.4 Duration of Therapy and Criteria for Removal from Study

Duration of treatment portion of the study (excluding screening time) will be approximately 85 Days (±7 days). By signing the informed consent form and agreeing to participate in this study, the subjects are required to participate in entirety up to the completion of all scheduled visits and study procedures, or until one of the following criteria applies:

- A protocol violation occurs
- Disease progression occurs
- A serious or intolerable adverse event occurs (that in the opinion of the Investigator, requires the subject's discontinuation)
- The Investigator withdraws the subject (at the Investigator's discretion for reasons other than the adverse event)
- The Principle Investigator terminates the protocol
- The subject requests to be discontinued from the protocol
- The subject is lost to follow–up
- Intercurrent illness that prevents administration of ^{225}Ac–J591
- Previous anaphylactic reaction to any J591 product

The investigators or physicians may stop the protocol or terminate a subject's participation in the protocol at any time should they judge:

- That it is not in the subject's best interest to continue
- If the subject experiences a protocol–related injury
- If the subject needs life–saving medications/procedures/treatment
- If the subject does not comply with the study plan
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

They may also remove the subject from the study for various other administrative and medical reasons. They can do this without the patient's consent.

6.5 Duration of Follow Up

Patients will be followed until death for survival assessment. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

7. DOSING DELAYS/DOSE MODIFICATIONS

There will be no dosing modifications. Unless there are specific reasons not to do so, all patients who are eligible for the trial will receive 5 ± 2 mCi (185 ±74 MBq) of ^{68}Ga−PSMA−HBED−CC during the screening visit and the Efficacy/Scan visit. During the treatment phase, the subject will receive single dose of ^{225}Ac−J591 based on the dose−level/Cohort to which he is assigned. The study chair must clear any dosing delays due to logistical issues (e.g. subject scheduling and/or radionuclide shipping that fall outside of the treatment window).

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with *Investigational Agent* can be found in **Section 13**.

8.1. Investigational Agent

A. ^{225}Ac−J591:

The humanized monoclonal antibody−huJ591 is provided by Dr. Neil Bander. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine. ^{225}Ac nitrate residue, 37 MBq (1.0 mCi) is supplied in a 2mL glass vial (by Department of Energy, Oak Ridge Laboratory), a radiochemical grade preparation. ^{225}Ac−DOTA−huJ591 mAb injection is manufactured by reacting ^{225}Ac chloride with DOTA−huJ591 (3.0 mg) aseptically withdrawn from the packaged drug substance vial and allowing the DOTA chelator to chelate ^{225}Ac in tetramethylammonium acetate buffer (TMAA). Following the reaction, the ^{225}Ac−DOTA−J591 is challenged with an excess of the chelator DTPA to remove any free or loosely bound Ac−225. ^{225}Ac−DOTA−huJ591 is then separated from ^{225}Ac−DTPA by gel filtration (Biogel P−6 column, Biorad, CA) using sterile saline solution containing 2% Human Serum Albumin as an eluent. The eluent fraction (4−8 mL) containing ^{225}Ac−DOTA−huJ591 is then sterilized by membrane filtration into a final drug product vial. QC Samples are removed for Quality Control. The specific activity of the ^{225}Ac−DOTA−huJ591 injection is estimated based on dose calibrator measurement of total ^{225}Ac activity (50 – 300μCi) and the total DOTA−huJ591 (3 mg) precursor used. Expected SA is 16.6 – 100 μCi/mg. Labeling efficiency and radiochemical purity will be determined using ITLC. Immunoreactivity of radiolabeled J591 mAb preparations will be determined using PSMA+ LNCaP tumor cells.

The material produced are subjected to quality assurance testing as outlined in the Food and Drug Administration "Points to Consider". All production data and supporting documents, together with the results of all quality assurance testing, have been provided to and reviewed by the Food and Drug Administration (^{225}Ac−J591, IND# **135267**).

B. ^{68}Ga−PSMA−HBED−CC:

PSMA−HBED−CC will be purchased from [redacted] and will be shipped to and stored at WCM as per manufacturer's guidelines. Upon subject's enrollment and confirmation of date of infusions, these peptides will be labeled with Gallium−68.

The material produced are subjected to quality assurance testing as outlined in the Food and Drug Administration "Points to Consider". All production data and supporting documents, together with the results of all quality assurance testing, have been provided to and reviewed by the Food and Drug Administration.

8.2. Availability

^{225}Ac−J591 (IND# **135267**) and ^{68}Ga−PSMA−HBED−CC (IND# 124495) are investigational agents supplied to investigators by Weill Cornell Medicine.

8.3. Agent Ordering

Upon enrollment of the subject, the WCM Study Coordinator will be notified about the ^{225}Ac−J591 and ^{68}Ga−PSMA−HBED−CC infusion dates. The WCM Study Coordinator will arrange with the staff of Nuclear Medicine Division at WCM for the timely labeling and delivery of the radiolabeled PSMA peptides to the site of infusion. The dates for study visits will be confirmed with the subject, site of infusion, and site of imaging.

8.4. Agent Accountability

The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of all agents received from Sponsor on a Drug Accountability Record Form (DARF).

9. CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 CTC count response

All subjects in this study will get blood samples drawn (at screening and EOS visit) for CTC enumeration by CellSearch methodology. Our primary analysis will assess those whose CTC counts drop to less than 5 or stay below 5 (responders) vs. those who remain at least 5 or above (non−responders) at EOS visit. We will also analyze the proportion with undetectable CTC count at 12 weeks and % changes from baseline to 12 weeks.

9.1.2 Cell−Free Plasma DNA:

Peripheral blood will be collected in two 10 mL Cell−Free DNA BCT® tubes (Streck) at the specified time points (screening or treatment,efficacy visit, and progression). A single sample for germline control will be collected in EDTA. These samples are collected for research purposes only and will not be billed to the subject. The cell−free plasma DNA will be analyzed for genomic studies, including the identification of DNA repair alterations.

9.1.3 Archival tissue:

Archival tissue will be requested during the screening visit. Fifteen unstained slides containing tumor material from archival paraffin−embedded tissue should be obtained. If available, metastatic tissue is preferred to prostate biopsy/prostatectomy specimens. The tissue will be analyzed for PSMA expression and targeted next-generation DNA and RNA sequencing, including assessment of DNA damage repair and AR pathways. Results will be for research purposes only.

9.1.4 Immune correlates:

Serum samples will be obtained during screening prior to treatment and at the main efficacy visit for immune biomarkers and processed as per the lab manual. We will utilize seromics to assess changes in antigen recognition before and after 225Ac−J591 and will also explore the induction of immunogenic cell death with HMGB1 (serum) and calreticulin (CTC). Stool will be collected prior to treatment to assess microbiome. Results of these assays will not be billed to the patient and are for research purposes only.

9.2. Imaging Studies

Conventional Imaging studies as well as ^{68}Ga−PSMA−HBED−CC PET/CT will be performed as described in schedule as well as clinically indicated to assess disease response. In subjects enrolled in this study, the measurable disease response will be calculated using Response Evaluation Criteria in Solid Tumors (RECIST Version 1.1) with PCWG3 modifications.

As an optional study in consenting subjects, will undergo two scans with ^{68}Ga-PSMA (with a minimum time of 6 h and maximum time of 2 days between the two injections). This cohort of patients will be analyzed to ensure that the peak standardized uptake values (including SUV_{mean}, SUV_{max}, SUV_{peak}, (SUV_{peak} not applicable to brain) of the lesions in the same patient/lesion is within an acceptable range of repeatability (+/- 30%) as demonstrated by prior radionuclide repeatability studies such as FDG and FLT.

10. MEASUREMENT OF EFFECT

10.1 CTC count response

All subjects in this study will get blood samples drawn (at screening and EOS visit) for CTC enumeration by CellSearch methodology. Our primary analysis will assess those whose CTC counts drop to less than 5 or stay below 5 (responders) vs. those who remain at least 5 or above (non−responders) at EOS visit.

In addition, it appears that decreases in CTC counts with therapy are a favorable marker even if the count remains at least 5. We will also analyze % changes in CTC counts with at least 50% decline in CTC count from baseline considered a response at 12 weeks. Best response will also be analyzed (% increase or best % decrease at any point will also be reported in a waterfall plot).

As reported in phase III studies of men with mCRPC, a favorable CTC count and LDH level at 12 weeks as well as undetectable CTC count at 12 weeks has been associated with overall survival. We will report the proportion of subjects who have CTC count <5 and normal LDH at the efficacy (scan) visit time point.

10.2 Biochemical (PSA) response

PSA response will be determined by comparing the PSA levels after therapy to the baseline, pre−treatment PSA. Declines of ≥ 30% and 50% confirmed by a second PSA value ≥ 2 weeks later, will be reported. Subjects must not demonstrate clinical or radiographic (CT and/or MR) evidence of disease progression during this time period.

10.3 Duration of PSA response

Duration of PSA response is defined as the time from the first 25% PSA decline until the PSA value is confirmed to increase by 25% above the nadir, provided that the increase is at least 2 ng/mL above the nadir.

10.4 PSA Progression

PSA progression will be defined as a rise of > 25% above either the pretreatment level or the nadir PSA level (whichever is lowest). PSA must increase by > 2 ng/ml to be considered progression. Confirmation requires a second consecutive rising PSA at least 2 weeks apart.

10.5 PSA Stabilization

PSA stabilization is referred to as any set of PSA values that do not meet the criteria for PSA response or PSA progression.

10.6 Time to PSA Progression

Time to PSA progression is defined as the interval between initiating treatment until the PSA rises 25% above nadir provided that the increase is at least 2 ng/mL.

10.7 Change in lesion size

In subjects with measurable disease, complete response (CR) is defined as complete disappearance of all measurable and evaluable lesions by physical examination or imaging studies and normalization of PSA with no appearance of new lesions for > 1 month. Partial response (PR) is defined as a 30% or greater reduction in the sum longest uni−dimensional diameter of all measurable lesions. There may be no new lesions. Stable Disease (SD) is characterized by subjects who do not meet the criteria of PR and who are without signs of progressive disease for at least 1 month. Disease Progression (DP) is defined as a greater than 20% increase in the sum longest uni−dimensional diameters of the indicator lesions or the appearance of new lesions. Bone scan progression (evaluable disease only) is requires at least 2 new lesions seen on a scan subsequent to baseline followed by a repeat scan at least 6 weeks later with at least one new additional lesion.

Conventional Imaging studies (MRI, CT, Bone Scan) along with optional but recommended investigational images (^{68}Ga−PSMA−HBED−CC PET/CT imaging) will be performed during the study visits or as clinically indicated to assess disease response. In subjects enrolled in this study, the measurable disease response will be calculated using Response Evaluation Criteria in Solid Tumors (RECIST Version 1.1) with PCWG3 modifications.

Radiographic scans will be used to assess best overall response and radiographic progression based on modified RECIST criteria for soft−tissue lesions and protocol−specific criteria for bone lesions Baseline images should be taken during Screening as close as possible to, and never more than 28 days before study visit 1. Every effort must be made to ensure the same radiographic method is used before and after treatment at scheduled visits. Radiographic progression free survival will be evaluated based on these results.

Analysis of Efficacy:

Median bPFS, rPFS, and OS, including survival curves, will be estimated using Kaplan−Meier methodology. Greenwood's formula will be used to calculate 95% confidence intervals for the Kaplan−Meier estimates. Percent change in PSA from baseline will be described by mean/median and standard deviation/inter−quartile range, as appropriate, depending on the distribution of percent change from baseline. Modified RECIST response (i.e., CR, PR, and CR/PR proportions), CTC count response proportion, defined favorable LDH/CTC proportion, and associated 95% confidence intervals, will be estimated via binomial proportions.

All subjects will be offered optional but recommended baseline and subsequent ^{68}Ga−PSMA−HBED−CC PET/CT. We will analyze the data for associations with response to

treatment as well as survival by chi−square tests/Fisher's exact tests and log−rank tests, respectively. For pre/post imaging comparisons, McNemar's chi−square test and paired t−tests/Wilcoxon signed−rank tests will be used, as appropriate. We plan to report data for each individual study as well as across the studies.

10.9 Calreticulin Expression on CTCs

We will analyze for associations between calreticulin expression and response to treatment and survival using chi−square tests/Fisher's exact tests and log−rank tests, respectively. We plan to report data for each individual study as well as across the studies.

10.10 Patient reported outcomes

Initial bone−targeted β−emitters were approved for the control of painful bone metastases. Radium−223 dichloride appears to be associated with improved/preserved patient reported outcomes as well. We will assess pain prior to and following treatment with the brief pain inventory (and will also collect pain medication data). We will assess global and prostate cancer specific patient reported outcomes with BPI−SF and FACT−P questionnaire. As with the other correlative studies, we will plan to report results for this individual study as well as across current and prior studies, particularly examining subjects that receive what we believe are efficacious doses.

11. DATA REPORTING / REGULATORY CONSIDERATIONS

11.1. Data Collection

The data collection plan for this study is to utilize REDCap to capture all treatment, toxicity, efficacy, and adverse event data for all enrolled patients.

11.1.1. REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill−Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web−based data−entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC−owned servers that are backed up nightly and support encrypted (SSL−based) connections. Nationally, the software is developed, enhanced and supported through a multi−institutional consortium led by the Vanderbilt University CTSA.

11.2. Regulatory Considerations

All protocol amendments and consent form modifications will be made by the Principal Investigator. Should an external sponsor be identified, they will have the opportunity to review and approve the changes prior to submission of these changes to the local IRB and distribution to participating sites.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

This is a phase 1 study of subjects with documented progressive metastatic CRPC with the primary endpoint of determination of MTD (and recommended phase II dose). In general, if there are sufficient numbers of subjects, descriptive statistics (e.g., number of observations, means, standard deviations, medians, and ranges) will be used to summarize data and selected endpoints may be summarized by dosing regimen. Otherwise, subject listings will be provided. No formal hypothesis testing is planned.

The dose−escalation schedule (accelerated dose−escalation followed by 3+3 Fibonacci escalation). Definitions of DLT and determination of MTD are defined above (**Section 6.2.10**). In the initial accelerated dose−escalation portion, cohorts will consist of single−subjects. If no > grade 1 at least possibly related AE (or >1 grade increase for pre-existing AE) occurs within 8 weeks as judged by the investigator, AE grading and attribution will be reviewed by the study chair (and discussed with the medical monitor if there are questions about severity or attribution) and treatment in the next cohort will begin. In the event of any grade $\geq$ 2 attributable toxicity (excluding infusion reactions) OR enrollment in Cohort 5 (whichever is first), the study will transition to modified 3+3 dose escalation cohorts. In addition to determination of MTD, we wish to gain additional information at lower doses that appear to be efficacious and safe. The rationale is to better evaluate a recommended phase 2 dose based upon combination of efficacy and toxicity information (rather than just highest tolerable dose per DLT and 3+3 definition) and to also have additional information at lower doses that appear to have some efficacy that might be used in future combination studies. Therefore, we will "backfill" cohorts that have met both of the following criteria: i) 0 of 3 subjects with DLT and ii) at least 1 of 3 subjects with >30% PSA decline and/or measurable disease response. Cohorts of single-subjects are not eligible for expansion.

The overall design is constructed to reduce exposure to subtherapeutic doses of study drug. The design of the 3+3 dose-escalation stage is to reduce the chance of escalating the dose when the probability of DLT is high, and increase the chance of escalating the dose when the probability of DLT is low. The maximum tolerated dose is defined as the highest dose level with an observed incidence of DLT in no more than one out of six patients treated at a particular dose level. The dose escalation scheme provides the following probabilities of escalation based on the true chances of DLT at a specific dose level. One can see that the probability of escalation is high if the toxicity risks are low.

True Probability of Toxicity	0.05	0.10	0.20	0.30	0.40	0.50	0.60
Probability of Escalation	0.97	0.91	0.71	0.49	0.31	0.17	0.08

12.2 Sample Size/Accrual Rate

The planned sample size for this dose escalation Phase I study is 2−52 treated subjects and accrual rate will be 1 subject every 2−3 months during the accelerated dose−titration portion, then 1−2 patients/month during modified 3+3 dose−escalation and expansion cohorts.

Sample size determination for the dose-escalation portion of the study is as described above. Sample size determination for the expansion cohort is as follows. We will enroll up to 16 subjects into the expansion cohort (including the 3-6 used for establishing the MTD and 10 others for additional safety information). We will define evaluable patients as patients who met eligibility requirements, have initiated therapy, and were not removed from the study for non-compliance or patient withdrawal.

Sample size recommendations for the two-stage design are determined according to Simons two-stage minimax design. We project a 30% PSA decline proportion of 20%, below which the regimen will be unacceptable and a 30% PSA decline proportion of 40%, above which the regimen will be considered worthy of further exploration. The null hypothesis that the 30% PSA decline proportion is less than or equal to 20% will be tested against the alternative hypothesis that the 30% PSA decline proportion is greater than or equal to 40%.

The sample size computations were performed assuming a 10% level of significance and 80% power. If 1 or fewer of the first 9 evaluable patients do not experience a 30% decline in PSA (stage 1), the study will be terminated and declared to have a negative result. If 2 or more patients out of the first 9 evaluable patients experience a 30% decline in PSA, ongoing accrual will proceed to the target sample size of 16 patients (stage 2). The new regimen will be declared effective and worthy of further testing if 5 or more patients experience a 30% PSA decline among the 16 patients entered. This two-stage design yields a 0.80 probability of a positive result if the true 30% PSA decline proportion is 40%. It yields a 0.90 probability of a negative result if the true 30% PSA decline proportion is 15%.

12.3 Stratification Factors

There are no planned stratification factors. Descriptive statistics will be utilized. Stopping rules for futility are in place as per **Section 4.2**.

12.4 Analysis of Endpoints

12.4.1 Analysis of Primary Endpoints

The primary endpoint will be the proportion of subjects with DLT from Visit 1 through End of Study Visit. The MTD is the highest dose amongst the different dose−level cohorts in this study at which no more than 2 (33%) of the subjects in a cohort experience DLT.

12.4.2 Analysis of Secondary Endpoints

For PSA response, CTC response and Imaging response, Descriptive analysis will be done. For imaging response RECIST criteria with PCWG3 modifications will be applied.

12.5 Interim Analysis

No interim analysis is planned (though safety analyses will occur in real−time and prior to each dose−escalation)**.**

12.6 Reporting and Exclusions

12.6.1 Evaluation of toxicity

All subjects will be evaluable for toxicity from the time of their first infusion with ^{68}Ga−PSMA−HBED−CC as well as when they receive their treatment single dose of with ^{225}Ac−J591. The distributions of the maximum observed grade toxicity will be tabulated for each type of toxicity and presented by dose level and overall. Results will be summarized with descriptive statistics.

12.6.2 Evaluation of response

Subjects who complete ^{225}Ac−J591 treatment and at least 12 weeks of subsequent follow−up evaluations (as described in the Schedule Calendar−**Section 6.1**) will be considered evaluable (consistent with PCWG3 guidelines). As the timing of response is not known, should the anticipated time to response be determined to be earlier than with other therapies using alternative markers of response, we may not replace subjects who are not evaluable through week 12 on study**.**

13. ADVERSE EVENT REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safe use of the drug or device under investigation. Safety will be monitored by evaluation of adverse events reported by patients or observed by investigators or research staff, as well as by other investigations such as clinical laboratory tests, x−rays, electrocardiographs, etc.

13.1 Adverse Event Definition

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off−label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

13.1.1. Investigational Agent Risks

There are no known contraindications for ^{68}Ga−PSMA−HBED−CC or ^{225}Ac−J591. Because of the potential for infusion or allergic reaction, the subject should be monitored for safety for one hour following the infusion.

Based on prior studies, the known side effects, risks, and hazards associated with the administration of ^{225}Ac−J591 include: infusion reaction (fever, chills, rash, hypotension, and/or hypertension after injection), hematological/bone marrow toxicity (thrombocytopenia, neutropenia), pain,and transient hepatic enzyme elevations. In addition, allergic reactions including anaphylaxis, renal failure, and CNS toxicity are a possibility. Based upon experience with another 225Ac radiolabeled antibody (for leukemia), we will administer saline and instruct subjects to increase oral hydration for the week following treatment to reduce the risk of non-specific renal toxicity from daughter radionuclides.

Precautions/monitoring:

The infusion of ^{68}Ga−PSMA−HBED−CC or ^{225}Ac−J591 and subsequent monitoring will occur in a facility that is equipped for cardio–pulmonary resuscitation. The dispensed dose will be infused under the supervision of nuclear medicine physician or designee under the supervision of a nuclear medicine physician. Infusion–related reactions (fever, rigors) will be treated with acetaminophen, meperidine and diphenhydramine hydrochloride as clinically appropriate. A minimum of 1 liter normal saline will be administered before/during/after ^{225}Ac−J591 infusion. Vital signs will be monitored before/after the ^{68}Ga−PSMA−HBED−CC or ^{225}Ac−J591 infusion. Systolic and diastolic blood pressure (mm Hg), temperature, pulse rate (beats/minute), and respiratory rate (breaths/minute), will be recorded with the patient in sitting position. Any clinically significant change in the vital signs will be recorded as AEs. Serial vital signs including temperature, BP, and heart rate will be monitored within 30 minutes of the ^{225}Ac−J591 infusion, and within 30 minutes, 60 (+/− 10) minutes, 90 (+/− 10) minutes, and 120 +/− 10 minutes after the^{225}Ac−J591 infusion. If any subject has any adverse reaction within 120 minutes for ^{225}Ac−J591, they will stay longer until it is resolved.

13.1.2. Adverse Event Characteristics and Related Attributions

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be

utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Attribution of the AE:

Definite – The AE *is clearly related* to the study treatment. A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction can be confirmed with a positive re–challenge test or supporting laboratory data.

Probable – The AE *is likely related* to the study treatment. A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the patient's clinical state or other modes of therapy administered to the patient.

Possible – The AE *may be related* to the study treatment. A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the patient's clinical state or other modes of therapy administered to the patient.

Unlikely – The AE *is doubtfully related* to the study treatment. The current state of knowledge indicates that a relationship is unlikely.

Unrelated – The AE *is clearly NOT related* to the study treatment. No relationship between the experience and the administration of study drug; related to other etiologies such as concomitant medications or patient's clinical state.

13.1.3. Recording of Adverse Events

All adverse events will be recorded on a patient specific AE log. The AE log will be maintained by the research staff and kept in the patient's research chart.

13.1.4. Reporting of AE to WCM IRB

All AEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link:

[redacted]

13.2. Definition of SAE

SAE's include death, life threatening adverse experiences, hospitalization or prolongation of hospitalization, disability or incapacitation, overdose, congenital anomalies and any other serious events that may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition.

13.2.1. Reporting of SAE to IRB

All SAEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link:

[redacted]

13.2.2. Reporting of SAE to FDA

If an SAE occurs on this study, the event will be filed on a MedWatch form with the FDA. The investigator must notify the FDA of any SAE's as soon as possible but no later than 7 calendar days after the initial receipt of the information

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
5901−B Ammendale Road
Beltsville, MD 20705−1266

13.3. AE/SAE Follow Up

All SAEs and AEs reported during this study will be followed until resolution or until the investigator confirms that the AE/SAE has stabilized and no more follow−up is required. This requirement indicates that follow−up may be required for some events after the patient discontinues participation from the study.

14. DATA AND SAFETY MONITORING PLAN (DSMP)

This study will utilize the Weill Cornell Medicine (WCM) Institutional Data Safety Monitoring Board (DSMB) and follow its policies and procedures for monitoring this study for safety concerns, with ongoing updates from the Study Chair on a continuous basis.

The Weill Cornell's DSMB is comprised of medical specialists and advisors on human rights issues in human subjects research. The DSMB currently has 9 members, meets at quarterly

intervals during the year, and carries out ongoing review of protocols submitted throughout the year. Once a protocol has been submitted and approved by the Institutional Review Board (IRB) and is recommended for oversight by the DSMB, the Board determines if the protocol will be reviewed quarterly, semi−annually, or annually.

The DSMB evaluates the accumulated data from the study in order to monitor the safety of subjects throughout the trial and reviews the risks and benefits, as well as the efficacy, of the study. The DSMB will also evaluate the overall trial conduct and progress. Ultimately, the DSMB validates the continuation of the trial or determines if a study needs modification or termination.

Reports to the DSMB will include the following items for review:

- Completed DSMB Periodic Review Form.
- Synopsis of the study to date.
- IRB approved consent form.
- IRB current protocol.
- Summary table of study results.
- Adverse event table.
- Data safety monitoring plan.

Safety monitoring is carried out to ensure and maintain the scientific integrity of human subject research projects and to protect the safety of human subjects. Safety monitoring can be viewed as any process during a clinical trial that involves the review of accumulated outcome data for groups of patient−subjects to determine if any of the treatment procedures practiced should be altered or stopped. NIH Guidelines (1998, 2000) specify that all clinical trials should have a system in place for appropriate oversight and monitoring to ensure the safety of participants and the validity of the data.

Monitoring activities will be commensurate with the nature, size, and complexity of the trial in accordance with institutional policies and will be determined after IRB and DSMB review of the protocol immediately prior to study activation. For a small, single−center study, usually a statistician in conjunction with a Safety Officer performs the monitoring. For that single−site, high−risk trials, a DSMB may be appropriate. For larger, single or multi−site studies, a committee, often called a Data Safety Monitoring Board (DSMB), usually performs the monitoring. Ongoing review of the data by an independent individual or committee assures the investigators, the IRB, the study's sponsor, and the funding agency that the trial can continue without jeopardizing subjects' safety.

Weill Cornell Medicine requires that all research approved by the WCMC IRB include an appropriate plan for the monitoring of data to ensure the safety of human subjects. Research supported by Federal agencies will be monitored according to all regulations and guidelines of the relevant Federal agency.

For this study, the DSMB will be notified after each cohort has been completed prior to dose escalation to the next cohort. In addition, a report will be made to the DSMB every 6 months.

14.1 Medical Monitor

The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor must also indicate whether he/she concurs with the details of the report provided by the principal investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death must be promptly forwarded to the appropriate committees/agencies. This individual will be a qualified physician, other than the principal Investigator, not associated with this particular study, able to provide medical care to research subjects for conditions that may arise during the conduct of this study, and will monitor the subjects during the conduct of the study.

Ashish Saxena, MD will serve as the Medical Monitor for this study.

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APPENDICES

APPENDIX A

Performance Status Criteria

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

APPENDIX B

WCMC IRB SAE Reporting Forms

[redacted]