



TITLE: Phase I/II dose-escalation trial of combination fractionated-dose ¹⁷⁷Lu-J591 and ¹⁷⁷Lu-PSMA-617 in patients with metastatic castration-resistant prostate cancer

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Confidentiality Statement

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List of Abbreviations

| | |
|-------------------------|---|
| 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 |
| ⁶⁸Ga | 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 |
| ¹⁷⁷Lu | 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 |
| ²²³Ra | 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/II dose-escalation trial of combination fractionated dose ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in patients with metastatic castration-resistant prostate cancer |
| Short Title: | ^{177}Lu -J591 and ^{177}Lu -PSMA-617 combination for mCRPC |
| Clinical Phase: | Phase I |
| Principal Investigator: | Scott T. Tagawa, MD, MS |
| Sample Size: | $N = 6 - 57$ (Phase I: 3 + 3 dose-escalation study design up to 6 dose-escalation / de-escalation cohorts for minimum of 6 and maximum of 30; Phase II minimum of 16 and maximum of 27) |
| Accrual Ceiling: | This study will enroll up to 57 subjects who receive treatment. |
| Study Population: | Adult male patients of ≥ 18 years with documented progressive metastatic CRPC |
| Accrual Period: | 3 years (approximately 1-2 patients per month with gaps between dose-escalation cohorts and between stage 1 and 2 of the phase II portion). |
| Study Design: | Phase I dose escalation study with combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 using a dose-fractionated regimen will be performed in patients with documented progressive metastatic CRPC. <u>The cumulative ^{177}Lu-J591 dose for each subject will be 2.7 GBq/m² (73 mCi/m²) of ^{177}Lu with 20 mg J591 and the cumulative ^{177}Lu-PSMA-617 dose for each subject will vary (depending on the Cohort) from 3.7 GBq (100 mCi) to 18.5 GBq (500 mCi).</u> The ^{177}Lu -PSMA-617 dose will be escalated in up to 6 different dose levels (3+3 dose-escalation study / de-escalation design). For the phase II portion, a minimum number of 14 patients will be enrolled at MTD (including those enrolled at MTD in Phase I) and a maximum of 24. |
| Study Duration: | Approximately 3 months after enrollment, then transition to long-term follow up |
| Study Agent: | 1) ^{177}Lu -PSMA-617 [1.85 GBq (50 mCi) – 9.25 GBq (250 mCi)] x2 doses, 2 weeks apart (Treatment Visit #1 and #2), IV administration 2) ^{177}Lu -J591 [1.35 GBq/m ² or 36.5 mCi/m ²] x2 doses, 2 weeks apart (Treatment Visit #1 and #2), IV administration 3) ^{68}Ga -PSMA-HBED-CC [185 \pm 74 MBq or 5 \pm 2 mCi] intravenous during screening and at 12 weeks (\pm 1 week) with standard imaging |
| Primary Objectives: | <ul style="list-style-type: none">(Phase I) Determine the dose limiting toxicity (DLT) of dose-fractionated combination therapy with ^{177}Lu-J591 and ^{177}Lu-PSMA-617 |

- (Phase I) Determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of dose-fractionated combination therapy with ^{177}Lu -J591 and ^{177}Lu -PSMA-617
 - (Phase II) To assess the proportion with PSA decline following the dose-fractionated combination therapy with ^{177}Lu -J591 and ^{177}Lu -PSMA-617
- Secondary Objectives:**
- To assess radiographic response rate by RECIST 1.1 with PCWG3 modifications
 - To assess biochemical and radiographic progression-free survival by PCWG3 criteria
 - To assess overall survival following the dose-fractionated combination therapy with ^{177}Lu -J591 and ^{177}Lu -PSMA-617
 - To assess safety of dose-fractionated combination therapy with ^{177}Lu -J591 and ^{177}Lu -PSMA-617 as assessed by CTCAE 4.0
 - To assess changes in CTC count as measured by CellSearch and the rate of favorable CTC count and LDH at 12 weeks following dose-fractionated combination therapy with ^{177}Lu -J591 and ^{177}Lu -PSMA-617
 - 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 immune effects of PSMA-targeted radionuclide therapy
 - To assess genomic alterations in relationship to outcome following dose-fractionated combination therapy with ^{177}Lu -J591 and ^{177}Lu -PSMA-617
 - To estimate whole body distribution and radiation dosimetry of ^{177}Lu -J591 and ^{177}Lu -PSMA-617
- Endpoints:** Dose limiting toxicity (DLT), adverse event rate, Maximum tolerated dose (MTD), recommended phase II dose (RP2D), PSA decline rate, response rate, and progression free survival.

Schema

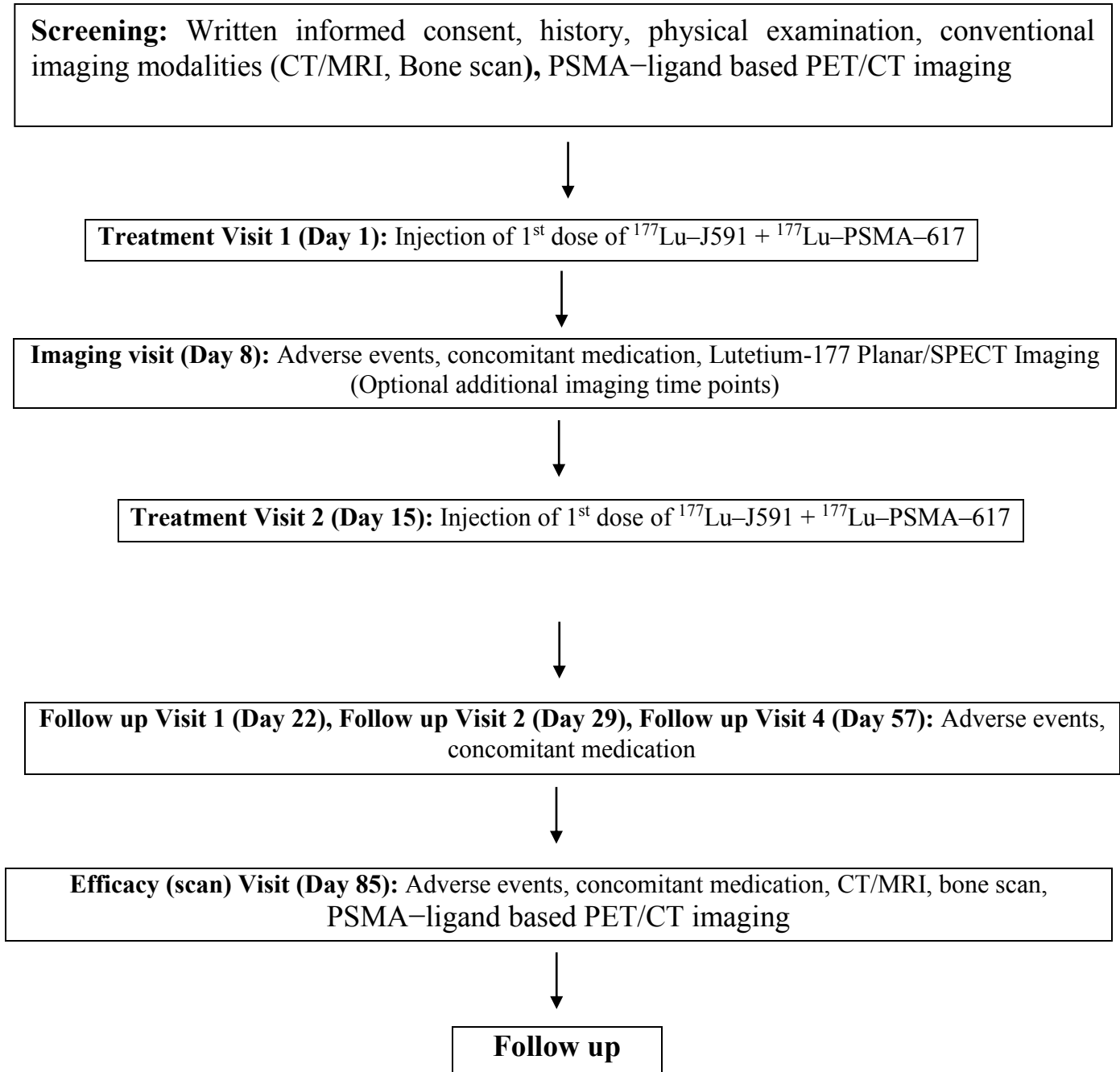


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1. STUDY OBJECTIVES

The objectives of this clinical trial are as follows:

1.1 Primary Objectives

- (Phase I) Determine the dose limiting toxicity (DLT) of the combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in a 2-week dose-fractionation regimen
- (Phase I) Determine the cumulative maximum tolerated dose (MTD) and/or recommended phase II dose (RP2D) of the combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in a 2-week dose-fractionation regimen
- (Phase II) To assess the proportion with PSA decline following treatment with the combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in a 2-week dose-fractionation regimen

1.2 Secondary Objectives

- To assess radiographic response rate by RECIST 1.1 with PCWG3 modifications
- To assess biochemical and radiographic progression-free survival by PCWG3 criteria
- To assess overall survival following treatment with the combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in a 2-week dose-fractionation regimen
- To assess safety of treatment with the combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in a 2-week dose-fractionation regimen as assessed by CTCAE 4.0
- To assess changes in CTC count as measured by CellSearch and the rate of favorable CTC count and LDH at 12 weeks following treatment with the combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in a 2-week dose-fractionation regimen
- 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 immune effects of PSMA-targeted radionuclide therapy
- To assess genomic alterations in relationship to outcome following treatment with the combination of ^{177}Lu -J591 and ^{177}Lu -PSMA-617 in a 2-week dose-fractionation regimen
- To estimate whole body distribution and radiation dosimetry of ^{177}Lu -J591 and ^{177}Lu -PSMA-617

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 immuno therapy 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 ¹¹¹Indium (¹¹¹In) and ¹⁷⁷Lutitium (¹⁷⁷Lu) 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 Actinium-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 ¹¹¹In using a DOTA chelate showed that

repetitive dosing was well tolerated with total doses of up to 500 mg/m² 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 initially performed at WCMC using a single-dose of ¹⁷⁷Lu or ⁹⁰Y 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, pharmacokinetics, 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 ¹⁷⁷Lutetium-Labeled J591 in subjects with metastatic castration-resistant prostate cancer (CRPC)(28):

¹⁷⁷Lu-J591 was evaluated in patients with metastatic CRPC demonstrating acceptable toxicity (MTD=70 mCi/m²), excellent targeting of metastatic sites and biologic activity. Thirty-five subjects received ¹⁷⁷Lu-J591, of whom 16 received up to three doses. Myelosuppression was dose limiting at 75mCi/m², and the 70mCi/m² dose level was determined to be the single-dose MTD. Repeat dosing at 45 to 60mCi/m² was associated with dose-limiting myelosuppression; however, up to three doses of 30mCi/m² 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 ¹⁷⁷Lutetium (¹⁷⁷Lu) radiolabeled J591 (¹⁷⁷Lu-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 ¹⁷⁷Lu-J591 in two cohorts (65 and 70mCi/m²). 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 ¹⁷⁷Lu 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/m², based upon FDA restrictions, the initial cohort was treated at 65 mCi/m². In an exploratory analysis, there was a dose–response relationship. The ≥30% PSA response rates in the 65 mCi/m² and 70 mCi/m² 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 ¹⁷⁷Lu–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 ¹⁷⁷Lu–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 ¹⁷⁷Lu–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 ¹⁷⁷Lu–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 ¹⁷⁷Lu–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 heme toxicities; 19 (57.6%) had Grade 4 heme 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 ¹⁷⁷Lu–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 ¹⁷⁷Lu–J591 Study and Phase 1 fractionated ¹⁷⁷Lu–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 ¹⁷⁷Lu–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 ¹⁷⁷Lu. Based upon this theory, a phase I trial of docetaxel and prednisone with escalating doses of fractionated ¹⁷⁷Lu–J591 was initiated. 15 men with median age 69.1 (49.3–80.8) were enrolled. The MTD/RP2D of ¹⁷⁷Lu–J591 was 40 mCi/m²x2 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 Radionuclide–chelating PSMA ligand

Targeted radionuclide therapy (both monoclonal antibody and/or urea based peptides) is a state of the art and rapidly developing therapy option for different cancer types. The potential advantage of targeted radionuclide therapy is saving the normal tissue while giving a high radiation dose to the tumor.

After rather unsuccessful therapy with ^{90}Y –CYT–356 monoclonal antibody (mAb) that binds to the intracellular domain of PSMA(43), phases 1 and 2 clinical trials utilizing the PSMA mAb J591, radiolabeled with ^{90}Y (Yttrium–90) or ^{177}Lu (Lutetium–177), have shown promising results(28, 34, 35, 39).

Also, PSMA has recently been discovered as a promising target for radioisotope based approaches, both for therapy, for example, using ligands labeled with either β – or α –emitting nuclides such as ^{131}I (Iodine–131), ^{177}Lu (Lutetium–177), and ^{225}Ac (Actinium–225), and for PET imaging, for example, using ^{68}Ga –labeled PSMA ligands. Despite encouraging early results, data about intended and unintended effects of PSMA–based radioligand therapies (RLTs) are still scarce.

Results from selective clinical trials using radiolabeled PSMA ligand for therapy and diagnosis of prostate cancer are reported here under:

2.4.1 ^{177}Lu –PSMA–617

a) ^{177}Lu –DKFZ–PSMA–617 therapy in metastatic castration resistant prostate cancer (44):

Thirty–one mCRPC patients with progressive disease despite second–line hormonal therapy and/or docetaxel chemotherapy were recruited for the study. All underwent ^{68}Ga –PSMA–HBED–CC PET/CT, prior to therapy with ^{177}Lu –DKFZ–PSMA–617. The mean activity administered was 5.07 ± 1.85 GBq ranging over one to four cycles. Study reported decline in PSA from baseline. Based on biochemical response criteria, 25/31 had either complete response, partial response or stable disease while 6 continued to have disease progression. The mean ECOG performance status improved from 2.54 to 1.78 after therapy. Two patients experienced grade I and grade II hemoglobin toxicity each, while none experienced nephrotoxicity or hepatotoxicity.

b) ^{177}Lu –PSMA–617 as A Novel Therapeutic Option in Patients With Metastatic Castration Resistant Prostate Cancer (45):

28 consecutive patients with mCRPC who have exhausted conventional therapeutic options were treated with 1 or 2 cycles of ^{177}Lu –PSMA–617. Mean administered activity at first therapy was

5.92 ± 0.44 GBq of ¹⁷⁷Lu-PSMA-617 and 5.86 ± 0.73 GBq at the second therapy. All patients underwent ⁶⁸Ga-PSMA-PET/CT prior to therapy. Any PSA decline occurred in 59% and 75% of patients after 1 and 2 Cycles. Similarly, a PSA decline of 50% or greater occurred in 32% and 50%. Hematologic and renal parameters changed insignificantly; permanent xerostomia or other safety-related toxicity did not occur. The estimated median survival was 29.4 weeks, which was significantly longer than survival in the historical best supportive care group.

c) PSMA-Targeted Radionuclide Therapy of Metastatic Castration-Resistant Prostate Cancer with ¹⁷⁷Lu-Labeled PSMA-617 (46):

The authors reported their experience with ¹⁷⁷Lu-PSMA-617-targeted radionuclide therapy in a case series of mCRPC patients resistant to other treatments. Patients were screened with either ^{99m}Tc-MIP1427 SPECT/CT (500–700 MBq) or ⁶⁸Ga-PSMA-11 PET/CT (150 MBq ± 20%). 30 patients received 1 to 3 cycles of ¹⁷⁷Lu-PSMA-617 (3.7 to 6 GBq per cycle) at 2 months interval. While 21 had a PSA response, 13 had a >50% PSA decrease. After 3 cycles, 8 of the 11 patients achieved a sustained PSA response (>50%) for over 24 weeks, which also correlated with radiologic response (decreased lesion number and size). Acute hematological toxicity was mild. Diffuse bone marrow involvement was a risk factor for higher grade myelosuppression but could be identified by PSMA imaging in advance. Xerostomia, nausea, and fatigue occurred sporadically (<10%). Clearance of non-tumor-bound tracer was predominantly renal and widely completed by 48 hours. Safety dosimetry revealed kidney doses of approximately 0.75 Gy/GBq, red marrow doses of 0.03 Gy/GBq, and salivary gland doses of 1.4 Gy/GBq, irrespective of tumor burden and consistent on subsequent cycles. Mean tumor-absorbed dose ranged from 6 to 22 Gy/GBq during cycle 1.

d) Pre-therapeutic dosimetry of normal organs and tissues of ¹⁷⁷Lu-PSMA-617 in CRPC (47):

Seven patients were screened after receiving 75–150 MBq of ⁶⁸Ga-PSMA-11 intravenously. PET/CT images were acquired 45–60 min post-injection. All had intense tracer uptake at the lesions. They then received ¹⁷⁷Lu-PSMA-617 activity ranged from 185 to 210 MBq with a mean of 192.6 ± 11.0 MBq. The highest radiation estimated doses were calculated for parotid glands and kidneys. Calculated radiation-absorbed doses per megabecquerel were 1.17 ± 0.31 mGy for parotid glands and 0.88 ± 0.40 mGy for kidneys. The radiation dose given to the bone marrow was significantly lower than those of kidney and parotid glands (*p* < 0.05). The calculated radiation dose to bone marrow was 0.03 ± 0.01 mGy/MBq.

Table 2: Calculated radiation-absorbed doses (mGy/MBq ¹⁷⁷Lu-PSMA-617) of organs of each patient:

| Patient | Age | GS | PSA (ng/ml) | Parotid gland | Kidney | Bone marrow | Liver | Total body | Residence time (h) |
|---------|-----|----|-------------|---------------|--------|-------------|-------|------------|--------------------|
| 1 | 65 | 8 | 48 | 1.66 | 0.76 | 0.025 | 0.27 | 0.049 | 24.6 |

| | | | | | | | | | |
|------|------|-----|------|------|------|-------|------|-------|------|
| 2 | 62 | 9 | 110 | 0.96 | 1.66 | 0.048 | 0.46 | 0.094 | 46.3 |
| 3 | 57 | 8 | 19 | 0.94 | 0.98 | 0.037 | 0.23 | 0.058 | 49.4 |
| 4 | 65 | 7 | 89 | 1.48 | 0.52 | 0.021 | 0.25 | 0.035 | 27.6 |
| 5 | 63 | 9 | 235 | 1.07 | 0.69 | 0.058 | 0.34 | 0.098 | 62.0 |
| 6 | 65 | 9 | 78 | 1.25 | 1.03 | 0.030 | 0.22 | 0.057 | 26.5 |
| 7 | 70 | 8 | 19 | 0.80 | 0.51 | 0.022 | 0.18 | 0.036 | 28.7 |
| Mean | 63.9 | 8.3 | 85.4 | 1.17 | 0.88 | 0.034 | 0.28 | 0.061 | 37.9 |
| SD | 3.9 | 0.8 | 74.5 | 0.31 | 0.40 | 0.014 | 0.09 | 0.026 | 14.6 |

*GS Gleason score, PSA prostate-specific antigen

Table 3: Maximum amount of radioactivity (GBq) to reach radiation-absorbed dose limits:

| Patient | Parotid gland (30 Gy) | Kidney (23 Gy) | Bone marrow (2 Gy) | Liver (32 Gy) | Total Body (2 Gy) |
|---------|-----------------------|----------------|--------------------|---------------|-------------------|
| 1 | 18.1 | 30.4 | 79.6 | 116.4 | 41.2 |
| 2 | 31.1 | 13.8 | 41.9 | 69.7 | 21.4 |
| 3 | 31.8 | 23.5 | 54.2 | 137.6 | 34.6 |
| 4 | 20.3 | 44.0 | 95.7 | 126.3 | 57.0 |
| 5 | 28.0 | 33.2 | 34.3 | 95.0 | 20.3 |
| 6 | 24.0 | 22.4 | 66.0 | 142.4 | 34.9 |
| 7 | 37.5 | 44.9 | 89.7 | 181.3 | 56.2 |
| Mean | 27.2 | 30.3 | 65.9 | 124.1 | 37.9 |
| SD | 6.9 | 11.5 | 23.7 | 35.7 | 14.8 |

*Organ radiation-absorbed dose constraints in parentheses

e) ¹⁷⁷Lu-PSMA Radioligand Therapy of mCRPC: Safety and Efficacy (48):

This study analyzed the safety and efficacy of the ¹⁷⁷Lu-labeled DOTAGA-based PSMA ligand ¹⁷⁷Lu-DOTAGA-(I-y)fk(Sub-KuE) (¹⁷⁷Lu-PSMA) in patients with mCRPC. 56 patients were enrolled and treated with ¹⁷⁷Lu-PSMA. ⁶⁸Ga-PSMA-(N,N'-bis-[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-N,N'-diacetic acid) (⁶⁸Ga-PSMA) PET/CT was used for patient selection and follow-up after therapy. ¹⁷⁷Lu-PSMA demonstrated high absorbed tumor doses (median 3.3 mGy/MBq) compared with the levels in normal organs. Parotid glands received higher doses (1.3 mGy/MBq) than kidneys (0.8 mGy/MBq). All patients tolerated the therapy without any acute adverse effects. Except for mild reversible xerostomia in 2 patients, no long-term side effects were observed. A decrease in PSA levels was noted in 45 patients (80.4%). Of the 25 patients monitored for at least 6 months after 2 or more therapy cycles, ⁶⁸Ga-PSMA PET/CT revealed partial remission in 14, stable disease in 2, and progressive disease in 9 patients. Contrast-enhanced CT revealed partial remission in 5, stable disease in 13, and progressive disease in 7 patients. The median progression-free survival was 13.7 months, and the median overall survival was not reached during follow-up for 28 months.

f) German collaborative publication

The largest published experience is a retrospective case series of 145 patients with mCRPC treated at 12 centers in Germany between February, 2014 and July, 2015(49). While not a prospective research study, in general the patients included men who had tumor progression despite abiraterone (64%) and/or enzalutamide (52%) and had either received chemotherapy (54%) or were unfit/refused chemotherapy. Patients also received radium-223 if able/eligible (17%). Treatment was administered only if PSMA uptake was demonstrated on PSMA imaging. On average, 5.9 GBq was administered per dose (range 2–8 GBq). Retreatment was allowed at physician discretion. Of assessable patients (n=99), 60% had PSA decline after initial treatment, with at least 50% PSA decline occurring in 40%. Safety was assessed by retrospective chart review with charts available in all 145 and labs available in 121 (83.4%). Grade >2 hematologic AEs occurred in 12% (grade 3/4 anemia in 10%, grade 3/4 leukopenia in 3%, and grade 3/4 thrombocytopenia in 4%). Other AEs of any grade that occurred $\geq 4\%$ include AST elevation 19%, fatigue 13%, renal failure 12%, ALT elevation 8%, xerostomia 8%, nausea 6%, dysgeusia 4%. While retrospective in nature, this represents the largest published series and demonstrates some biochemical anti-tumor activity without a high occurrence of high-grade adverse events.

g) Preliminary data from Australian prospective phase II study

Preliminary results of the first prospective study utilizing ^{177}Lu -PSMA-617 were reported at the 2017 ESMO annual meeting. ⁴⁶ Australian investigators enrolled 43 men with mCRPC and treated 30 (7 excluded due to imaging results, 6 others for eligibility issues). Following PSMA and FDG PET/CT, subjects received 4-8 GBq of ^{177}Lu -PSMA-617 and were planned to receive 4 doses every 6 weeks. Seventeen (57%) had at >50% PSA decline with median PFS 6.3 and OS 12.7 months. Toxicity was deemed acceptable. In this prospective study, but adverse events were more common than previously reported in retrospective studies. Xerostomia (grade 1-2) was common at 63%, nausea 50%, vomiting 20%, and fatigue 17%. All cause grade 3/4 anemia occurred in 23%, neutropenia 10%, thrombocytopenia 27%, though attributable grade 3/4 anemia occurred in 7%, neutropenia 7%, and thrombocytopenia 13%.

Additional therapeutic studies using different radiolabeled PSMA ligands are summarized in the table below:

Table 4: ^{177}Lu -PSMA-617: Pilot studies of targeted radionuclide therapy:

| | Radioligand Therapy | N | Dose (GBq) | Total doses | Reference |
|-----|---|----|-------------|----------------------------------|----------------------------|
| 1. | ^{177}Lu -DKFZ-PSMA-617 | 1 | 7.4 | 1 dose | Kratochwil et al. 2015 |
| 2. | ^{177}Lu -DKFZ-PSMA-617 | 10 | 4.1 – 6.1 | 1 dose | Ahmadzedeifar et al. 2015 |
| 3. | ^{177}Lu -PSMA-617 | 7 | 0.19 – 0.21 | 1 dose | Kabasakal et al. 2015 |
| 4. | ^{177}Lu -PSMA I&T | 2 | 5.7 – 8 | 1 dose | Weineisen et al. 2015 |
| 5. | ^{177}Lu -DKFZ-PSMA-617 | 5 | 3.4 – 3.9 | 2 doses, 10 weeks apart | Delker et al. 2016 |
| 6. | ^{177}Lu -PSMA-617 | 30 | 3.7 – 6 | 1 – 3 dose(s), 2 months apart | Kratochwil et al. 2016 |
| 7. | ^{177}Lu -PSMA-617 | 9 | 5.28 – 5.77 | 1 dose | Hohberg et al. 2016 |
| 8. | ^{177}Lu -PSMA-617 | 7 | 5.5 – 7.4 | 1 dose | Das et al. 2016 |
| 9. | ^{177}Lu -PSMA-617 | 23 | 7.4 | 1 dose | Demir et al. 2016 |
| 10. | ^{177}Lu -PSMA-617 | 28 | 5.13 – 6.59 | 1 – 2 dose(s), 2 months apart | Rahbar et al. 2016 |
| 11. | ^{177}Lu -PSMA-617 | 82 | 5.4 – 6.4) | 1 dose | Rahbar et al. 2016 |
| 12. | ^{177}Lu -PSMA-617 | 31 | 1.1 – 5.5 | 1 – 4 dose(s) | Yadav et al. 2016 |
| 13. | ^{177}Lu -PSMA-617 | 1 | NA | 3 doses, 8 weeks apart | Schlenkhoff et al. 2016 |
| 14. | ^{177}Lu -PSMA-617 | 43 | 6 | 1 – 4 dose(s) | Zimbelmann et al. 2016 |
| 15. | ^{177}Lu -PSMA-617 | 5 | 5.8 – 7.4 | 1 dose | Chakraborty et al. 2016 |
| 16. | ^{177}Lu -PSMA-617 + ^{177}Lu -EDTMP | 1 | 1.85 + 1.3 | 1 dose each | Bal et al. 2016 |
| 17. | ^{177}Lu -PSMA I&T | 22 | 3.4 – 7.4 | 1 – 4 dose(s), 2 weeks apart | Heck et al. 2016 |
| 18. | ^{177}Lu -PSMA I&T | 56 | 3.6 – 8.7 | 1 – 5 dose(s) | Baum et al. 2016 |
| 19. | ^{177}Lu -PSMA I&T | 18 | 7.4 | 1 – 4 dose(s) | Okamoto et al. 2017 |
| 20. | ^{177}Lu -PSMA-617 | 15 | 3.7 – 6 | 2 doses | Fendler et al. 2017 |
| 21. | ^{131}I -MIP-1095 | 36 | 1.5 – 7.2 | 1 – 3 dose(s) | Afshar-Oromeih et al. 2017 |
| 22. | ^{177}Lu -PSMA-617 | 1 | 5.9 – 6.4 | 4 doses | Roll et al. 2017 |

h. Fractionated dose ^{177}Lu -PSMA-617

Based upon our prior experience with radiolabeled J591 (demonstrating dose and toxicity response with improved safety with dose fractionation) and the worldwide experience of radiolabeled small molecules, we initiated a dose-escalation study of fractionated dose ^{177}Lu -PSMA-617 in January, 2018. As of the time of current protocol writing, 4 cohorts have been treated with safety follow up (and the 5th / final planned cohort is enrolled), with administered/planned dose levels described in the Table below. As predicted based upon dosimetry estimations, **no dose-limiting toxicity has been observed to date, including all potential dose-levels to be utilized in this protocol.**

Table: Dose-escalation strategy of fractionated dose ¹⁷⁷Lu-PSMA-617 protocol

| Cohort | Treatment Dose mCi (GBq) | | Total dose mCi (GBq) | # Subjects (n) |
|--------|--------------------------|------------|-------------------------|-------------------|
| | Day 1 | Day 15 | | |
| -1 | 50 (1.85) | 50 (1.85) | 100 (3.7) | 0 |
| 1 | 100 (3.7) | 100 (3.7) | 200 (7.4) | 6 |
| 2 | 150 (5.55) | 150 (5.55) | 300 (11.1) | 6 |
| 3 | 200 (7.4) | 200 (7.4) | 400 (14.8) | 5 |
| 4 | 250 (9.25) | 250 (9.25) | 500 (18.5) | 6 |
| 5 | 300 (11.1) | 300 (11.1) | 600 (22.2) | pending |

2.4.2 ⁶⁸Ga-PSMA-HBED-CC (⁶⁸Ga-PSMA-11)

PSMA has recently been discovered as a promising target for radioisotope based approaches for PET imaging, for example, using ⁶⁸Ga-labeled PSMA ligands(50-55). 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(56, 57). One of these ligands, the small molecule Glu-NH-CO-NH-Lys-(Ahx)-[⁶⁸Ga(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(58, 59). PET/CT-imaging with ⁶⁸Ga-PSMA-11 has demonstrated this novel method as an important imaging modality for diagnosing recurrent PCa (50, 60-62). Perera et al., did systematic review of ⁶⁸Ga-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(63).

Results from selective clinical trials using ⁶⁸Ga-PSMA-HBED-CC to evaluate its diagnostic value are reported here under:

- i. **Diagnostic value of ⁶⁸Ga-PSMA-HBED-CC PET/CT imaging in the diagnosis of recurrent prostate cancer(61):**

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 $172.4 \text{ MBq} \pm 70.9$, 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 (64):**

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 (52):

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 (60):**

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

- ^{177}Lu -J591 (IND #11613)

i. J591

The J591 Mab is a deimmunized monoclonal antibody directed at the extracellular domain of human PSMA. J591 was derived from murine J591 using Biovation's (Aberdeen, Scotland) Deimmunisation technology in which individual amino acids in predicted B and T cell epitopes were replaced with other amino acids such that the epitope would no longer be recognized by the human immune system, thereby decreasing the likelihood of the development of an anti-mAb antibody response in humans (65). This results in a potentially non-immunogenic antibody, which can be administered to patients on multiple occasions over long periods without inducing an immune response. Furthermore, the deimmunized Mab additionally has been engineered to possess the effect of inducing antibody dependent cellular cytotoxicity (ADCC) with human immune effector cells. J591 is produced from NS0 cells by Lonza Biologics (Slough, UK). The molecular weight of J591 is approximately 147,000 daltons as determined by Matrix Assisted Laser Desorption Mass Spectrometry (MALDI-TOF). The naked antibody is formulated in a 50 mM sodium phosphate, pH 5.5, containing 100 mM sodium chloride and 2 mM EDTA at a nominal concentration of 5 mg/mL.

ii. DOTA–J591

In order to radiolabel J591 with β^- emitting radionuclides such as ^{177}Lu , the J591 antibody molecule is first conjugated to a macrocyclic chelating agent, 1,4,7,10–tetraazacyclododecane–N,N',N'',N'''–tetraacetic acid (DOTA) by direct coupling of one of the four carboxylic acid groups of DOTA to the primary amines present in the protein structure. The DOTA conjugated antibody is formulated in 0.3 M ammonium acetate, pH 7.0 at a nominal concentration of 8 mg/mL.

iii. Radiolabeled J591 (^{177}Lu –J591)

The DOTA–J591 antibody molecule is labeled with radiometal ^{177}Lu in 1.0 M ammonium acetate buffer at pH 7.0. The radiolabeled J591 complex is then purified using size–exclusion column chromatography. To avoid the effects of autoradiolysis on the antibody, reaction time is minimized and the radiolabeled antibody preparations are formulated in phosphate buffered saline containing not more than 1% Human Serum Albumin (HSA). All reagents used in the conjugation and purification of J591 are made with pyrogen–free water. Radiolabeled J591 is periodically tested for sterility and pyrogenicity.

Each patient dose will be supplied to the study site on the day of or day before treatment by the central radiopharmacy. The J591 mAb is a deimmunized monoclonal antibody directed at the extracellular domain of human PSMA. For additional details, see Section 1 and/or Investigator Brochure. Radiolabeled antibody preparations are formulated in phosphate buffered saline containing not more than 1% Human Serum Albumin (HSA). In order to radiolabel J591 with β^- emitting radionuclides, the antibody molecule is first conjugated to a macrocyclic chelating agent, 1,4,7,10–tetraazacyclododecane–N,N',N'',N'''–tetraacetic acid (DOTA) by direct coupling of one of the four carboxylic acid groups of DOTA to the primary amines present in the protein structure. The radiolabeling of J591 with ^{177}Lu is achieved by adding the radionuclide to the ammonium acetate buffered J591. To avoid the effects of autoradiolysis on the antibody, reaction time is minimized and the reaction mixture is purified with a size exclusion column prior to administration. All reagents used in the conjugation and purification of J591 are made from pyrogen–free water. Radiolabeled J591 is tested periodically for sterility and pyrogenicity.

iv. Pharmacokinetics

The pharmacokinetics of J591 antibody was studied in patients with prostate cancer using ^{111}In –J591 as a radiotracer. In a Phase 1 dose escalation study (see above) 4 groups of patients received different amounts of J591 antibody (25, 50, 100, 200 mg/m²) mixed with a trace amount of ^{111}In –J591 (5 mCi/1–2 mg). In a different Phase 1 dose–escalation study using ^{90}Y –J591 (see above), a group of patients received 20 mg of total J591 antibody mixed with a trace amount of ^{111}In –J591 (5 mCi/1–2 mg) for imaging studies prior to ^{90}Y treatment dose. These studies demonstrated that the plasma clearance kinetics of ^{111}In –J591 antibody is dose–dependent at low doses (<50 mg/m²). It should be noted that, in these studies, plasma clearance kinetics was determined based on the measurement of radioactivity in the plasma over time. The actual J591 antibody levels have not been measured; there is an assumption that the clearance of radiolabeled antibody does indeed

reflect the J591 antibody clearance. The plasma clearance kinetics, based on bi-exponential fitting of plasma-time activity curves indicate that the terminal half-life ($T_{1/2\beta}$) is about 44.2 ± 13.9 hrs at 11 mg/m^2 antibody dose and 59.3 ± 16.6 hrs at 25 mg/m^2 antibody dose. At higher doses of antibody mass, the plasma clearance is relatively slower, but stable. In summary, the plasma clearance of antibody increases at lower doses of antibody ($<25 \text{ mg/m}^2$). In addition, repeat administration of J591 antibody over a 4-week period does not affect plasma clearance of radiolabeled J591 in patients with prostate cancer (data not shown). In a Phase 1 dose-escalation study using ^{177}Lu -J591 (see above), a group of patients received J591 antibody mixed with a ^{177}Lu -J591 ($10\text{--}70 \text{ mCi/m}^2$ and total J591 10mg/m^2). The plasma clearance kinetics (shown in **Table-5**) demonstrate that there is no difference between ^{177}Lu and ^{111}In labeled J591 antibody.

Table-5: ^{177}Lu -J591 Vs. ^{111}In -J591: Plasma Clearance Kinetics:

| Pts. | mg/m ² | T _{1/2} α (hr) | T _{1/2} β (hr) | V _d at T ₀ | Clearance |
|-------------------|-------------------|-------------------------|-------------------------|----------------------------------|-----------|
| n=18 [#] | 10 | 3.61±5.70 | 46.8±12 | 3949±1067 | 72±34 |
| n=16 [*] | 11 | 2.37±1.94 | 44.2±14 | 4042±863 | 94±34 |
| n=3 [§] | 25 | 3.36±2.17 | 59.3±17 | 3698±224 | 56±16 |
| n=3 [§] | 50 | 7.83±9.5 | 99.7±32 | 4156±256 | 47±6.7 |
| n=3 [§] | 100 | 5.7±7.5 | 105±38 | 4373±566 | 40±13 |

[#] = In a Phase 1 Dose escalation protocol, 18 patients were treated with ^{177}Lu -J591

^{*} = In a Phase 1 Dose escalation protocol, 16 Patients were treated with ^{90}Y -J591 and one week prior to treatment, ^{111}In -J591 kinetics were studied.

[§] = In a Phase 1 Dose escalation protocol with huJ591 cold antibody treatment, 3 patients in each group received different amounts of cold huJ591 mixed with ^{111}In -J591.

v. Biodistribution

The imaging studies with ^{111}In -J591 in patients with prostate cancer showed that liver is the principal organ with significant uptake of radiolabeled antibody. The liver uptake of J591 is a function of J591 antibody mass administered (**Table-6**): the higher the antibody mass, the lower the relative proportional uptake of radiolabeled antibody by the liver. In addition, at 20 mg of J591 administered in Phase 1 dose escalation trials with ^{111}In -DOTA-J591, the liver uptake increases with time and is at a maximum by day 6 (27.0 ± 1.7 % of injected dose). No significant localization was seen in other major organs, such as spleen and kidneys.

Biodistribution: ^{111}In -J591 vs. ^{177}Lu -J591. The imaging studies were done 5 times over a period of one week with ^{111}In and 2 weeks with ^{177}Lu . The uptake of radioactivity in various source

organs (% injected dose/organ) was determined. The imaging studies clearly document that the in vivo distribution of radiolabeled J591 during the first week is quite similar with both ¹¹¹In and ¹⁷⁷Lu radionuclides (Table-7).

Table-6: ¹¹¹In-J591: Liver Uptake (% injected dose) as a Function of J591 Antibody Mass:

| <u>Time</u> | <u>11 mg/m²</u> | <u>25 mg/m²</u> | <u>50 mg/m²</u> |
|-------------|----------------------------|----------------------------|----------------------------|
| 1 | 13.1 ± 2.5 | 9.6 ± 1.0 | 7.9 ± 0.8 |
| 24 | 19.0 ± 3.2 | 12.9 ± 2.1 | 11.2 ± 1.2 |
| 48 | 20.8 ± 2.8 | ND | 11.0 ± 0.4 |
| 72 | 22.5 ± 2.4 | 14.5 ± 1.2 | 12.1 ± 1.0 |
| 120 | 24.6 ± 2.8 | ND | ND |
| 144 | 27.0 ± 1.7 | 17.1 ± 3.2 | 13.9 ± 1.0 |

Table-7: Biodistribution of Radiolabeled J591 Antibody:

| Organ | ¹¹¹In-J591 | | | ¹⁷⁷Lu-J591 | | |
|-----------------------|------------------------------|--------------------|--|------------------------------|-------------------|--|
| | DAY 0 | DAY 6 | | DAY 0 | DAY 7 | |
| Whole Body | 100.0 ± 0.0 | 66.1 ± 18.4 | | 100.0 ± 0.0 | 69.8 ± 9.5 | |
| Remainder | 52.3 ± 5.7 | 24.9 ± 16.8 | | 57.5 ± 6.4 | 34.7 ± 6.9 | |
| Heart Contents | 8.9 ± 2.3 | 3.4 ± 1.2 | | 8.8 ± 2.2 | 3.1 ± 0.9 | |
| Kidneys | 2.4 ± 0.8 | 2.5 ± 0.7 | | 2.2 ± 0.5 | 2.5 ± 0.5 | |
| Liver | 14.7 ± 3.2 | 28.0 ± 7.8 | | 15.0 ± 3.8 | 23.7 ± 7.0 | |
| Lungs | 9.0 ± 2.0 | 4.4 ± 1.7 | | 6.9 ± 1.9 | 3.1 ± 0.7 | |
| Red Marrow | 10.9 ± 5.0 | 1.2 ± 1.1 | | 7.4 ± 2.4 | 0.6 ± 0.4 | |
| Spleen | 1.8 ± 0.6 | 1.7 ± 0.8 | | 2.2 ± 1.0 | 2.1 ± 0.8 | |

vi. Radiation Dosimetry: ¹⁷⁷Lu-J591

Based on imaging studies and plasma clearance with ¹⁷⁷Lu-J591, the residence times (τ) of radioactivity in various source organs (as shown in Table-7) were calculated. Using “S” factors from MIRD tables, radiation dosimetry (cGy/mCi) for ¹⁷⁷Lu-J591 was estimated (Table-8). Following administration of ¹⁷⁷Lu-J591, the liver is the critical organ, receiving 7.77± 2.23 rads/mCi, and the kidneys and spleen receive <7 rads/mCi. The bone marrow dose was estimated to be 1.17± 0.37 rads/mCi based on the assumption that 36% of bone marrow volume represents plasma volume. Hematopoietic toxicity is usually the dose-limiting factor in RIT(66). Radiation dose of 9.0 Gy to the bone marrow is generally regarded as an “organ injury dose” and the tolerable

absorbed doses were usually estimated to be 65% of LD50 (50%) organ injury dose. Therefore, the tolerable absorbed dose for bone marrow is about 3.0 Gy (330 rads). The maximum tolerable radiation doses to kidneys, liver and spleen are approximately 20–25 Gy (2000–2500 rads). Dosimetric studies using radiolabeled antibodies have been effectively used by many investigators to determine patient-specific radiation dosimetry of radiolabeled antibody treatment(67, 68). Based on the dosimetry data shown in **Table–8**, the maximum bone marrow dose following administration of 70mCi/m² of ¹⁷⁷Lu–J591 would deliver 164 rads to an average person with a BSA of 2.0 m². The maximum dose the liver would be about 1100 rads, well below the maximum tolerable liver dose.

Table–8: Radiation Dosimetry (rads/mCi) of ¹⁷⁷Lu–J591 in Patients with Prostate Cancer:

| Organ | Mean | S.D. |
|------------------------------|-------------|---------------|
| Adrenals | 0.52 | ± 0.08 |
| Brain | 0.40 | ± 0.07 |
| Gall Bladder | 0.55 | ± 0.08 |
| LLI Wall | 0.42 | ± 0.07 |
| Small Intestine | 0.43 | ± 0.07 |
| Stomach | 0.45 | ± 0.07 |
| ULI Wall | 0.44 | ± 0.07 |
| Heart Wall | 3.50 | ± 0.70 |
| Kidneys | 5.20 | ± 1.29 |
| Liver | 7.77 | ± 2.23 |
| Lungs | 2.79 | ± 0.80 |
| Muscle | 0.42 | ± 0.07 |
| Pancreas | 0.52 | ± 0.08 |
| Red Marrow | 1.17 | ± 0.37 |
| Bone Surfaces | 0.70 | ± 0.14 |
| Skin | 0.42 | ± 0.07 |
| Spleen | 7.28 | ± 3.41 |
| Testes | 0.36 | ± 0.13 |
| Thymus | 0.44 | ± 0.07 |
| Thyroid | 0.41 | ± 0.07 |
| Urin. Bladder Wall | 0.97 | ± 0.22 |
| Total Body | 0.71 | ± 0.10 |
| Effective Dose Equiv. | 2.14 | ± 0.35 |
| Effective Dose | 1.33 | ± 0.19 |

- **⁶⁸Ga-PSMA-HBED-CC (IND # 124495)**

PSMA has recently been discovered as a promising target for radioisotope based approaches for PET imaging, for example, using ⁶⁸Ga-labeled PSMA ligands(50-55). 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(56, 57). One of these ligands, the small molecule Glu-NH-CO-NH-Lys-(Ahx)-[⁶⁸Ga(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(58, 59). PET/CT-imaging with ⁶⁸Ga-PSMA-11 has demonstrated this novel method as an important imaging modality for diagnosing recurrent PCa(50, 60-62). Perera et al., did systematic review of ⁶⁸Ga-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(63).

ABX Advanced Biochemical Compounds, Radeberg, Germany produces PSMA-HBED-CC for clinical use. Upon purchase and shipment to Weill Cornell Medicine, the peptide will be labeled with ⁶⁸Ga with the final product being ⁶⁸Ga-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 (⁶⁸Ga-PSMA-HBED-CC, IND# 124495).

i. Radiation Dosimetry: ⁶⁸Ga-PSMA-HBED-CC

Radiation dose estimates were provided by Professor Marcus Schwaiger of Technische Universitat Miiinchen (TUM), Munich, Germany. Balb1-c mice were used to determine the biodistribution at 4 time points after administration. Tissues were dissected after sacrifice and the percent of the administered dose was determined per organ and per gram of organ weight. The data of the biodistribution were extrapolated from mouse to man using OLINDA/EXM 1.0 Dosimetry Software (64; Stabin MG, et al. J Nucl Med. 2005 Jun;46(6):1023-7.).

Table 9: Radiation dose estimates for Humans administered 148 or 185 MBq (4 or 5 mCi) of 68Ga-PSMA-HBED-CC:

| Administered dose (Mbc) | 150 +/-20 | 185 +/-20 |
|-------------------------|-----------------|-----------------|
| Administered dose (mCi) | (4.0) | (5.0) |
| organ | [mSv] per organ | [mSv] per organ |
| Brain | 1.64E-01 | 2.05E-01 |
| Lung | 8.17E-01 | 1.02E+00 |
| Liver | 1.30E+00 | 1.63E+00 |
| Kidneys | 1.11E+02 | 1.39E+02 |
| Stomach Wall | 3.32E+00 | 4.15E+00 |
| Spleen | 7.96E+00 | 9.95E+00 |
| Pancreas | 2.80E+00 | 3.50E+00 |
| Red Bone Marrow | 1.35E+00 | 1.69E+00 |
| Bladder Wall | 1.41E+00 | 1.76E+00 |
| Effective Dose | 1.94E+00 | 2.43E+00 |

For comparison purposes, the average per capita annual exposure from natural background in the continental USA is 3.0 mSv. (*Ionizing radiation exposure of the population of the United States. Bethesda, Md.: National Council on Radiation Protection and Measurements. 2009. ISBN 978-0-929600-98-7. NCRP No. 160.*)

- **¹⁷⁷Lu-PSMA-617 (IND # 131966)**

Modifications of PSMA-11 have resulted in the development of another novel small molecule PSMA-ligand, PSMA-617. In preclinical studies, this ligand showed a significantly improved binding affinity to PSMA as well as a highly efficient internalization into PCa cells(69).

PSMA-617 can be labeled with radionuclides like ^{68}Ga (Gallium-68), ^{177}Lu (Lutetium-177), ^{111}In (Indium-111), and ^{90}Y (Yttrium-90) and, therefore, be used for PET-imaging as well as for radioligand-based therapy. Preclinical assays of PSMA-617 showed K_i values of 2.3 ± 2.9 nM, demonstrating a significant improvement compared to PSMA-11 (12.0 ± 2.8 nM). Based on these results, PSMA-617 has one of the highest binding affinities to the PSMA receptor, which have been published, so far. In preclinical studies, tumor-to-background ratios of up to 1,058 were observed at 24h post infusion. In addition, the internalization of the PSMA-617 into the PCa cells is highly effective: Internalized fraction: 17.67 ± 4.34 % IA/106 LNCaP cells (PSMA-11: 9.47 ± 2.56 % IA/106 LNCaP cells)(59, 69). Since 2013, ^{177}Lu -PSMA-617 has been increasingly used for radioligand therapy of metastatic PCa patients in several centers (Bad Homburg, Bonn, Cologne, Freiburg, Heidelberg, Istanbul, Melbourne, LMU Munich, Münster)(70).

Radiation dosimetry and organ specific dosage details are described in **section 2.4.1** and **Table 2 & 3** above. Toxicity data from various studies on ^{177}Lu -PSMA-617 is described in **section 2.7 Table 12**.

Diagnostic evaluation with ^{68}Ga -PSMA-HBED-CC followed by therapy with ^{177}Lu -PSMA-617 is thus opening a new theranostic potential of nuclear medicine. Initial studies in patients have demonstrated the utility of ^{177}Lu -PSMA-617 for the treatment of disseminated prostate cancer(70, 71). Dosimetric evaluation has shown that the critical organs are the parotid glands, and that up to 27 GBq (~700 mCi) of ^{177}Lu -PSMA-617 can be administered without crossing the 30 Gy critical dose threshold to the parotid glands(47).

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 (72). However, even after surgical treatment, high risk PC is prone to recur and often becomes resistant to conventional hormonal therapy (73). For this reason, efforts have been made to optimize patient outcome in the adjuvant and neoadjuvant settings (74-76). Thus far, neoadjuvant therapies have yet to demonstrate a survival benefit (77). 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 (78). 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 (79), 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, 80, 81).

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 (82). Another clinically validated target is human epidermal growth factor receptor 2 (HER2), which in breast cancer is targeted by trastuzumab (83). 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, 84-88).

A. Rationale for the appropriate radioisotope:

As described earlier, targeted radionuclide therapy as a treatment choice for mCRPC patients is being actively studied at various centers in the world (especially Europe and Australia). When compared to monoclonal antibodies, the low molecular weight compounds have a higher permeability into solid tumors, offer a significant advantage in achieving higher tumor uptake as well as a high percentage of specific binding. Additionally, small molecules display more rapid tissue distribution and faster blood clearance compared with intact immunoglobulins. These properties often lead to an enhanced target to non–target tissue ratio, which is important not just for imaging but also for successful application of therapeutic absorbed doses.

Following tumor localization, the radiometal is trapped within the cell, leading to higher accretion of radionuclide by the tumor. ^{131}I has lower energy β^- particles and longer physical half–life (max β^- 0.61 MeV; $T_{1/2} = 8.04$ d) compared to that with ^{90}Y (max β^- 2.28 MeV; $T_{1/2} = 2.67$ d). Because of its higher energy, ^{90}Y may be appropriate for larger tumors while ^{131}I may be more cytotoxic for smaller, micro–metastatic lesions. Since ^{90}Y does not emit any γ photons, the corresponding ^{111}In labeled antibodies are generally used as chemical and biological surrogates to study biodistribution and estimate radiation dosimetry of ^{90}Y labeled antibodies. In addition to ^{90}Y , the lanthanide radiometal, ^{177}Lu (max β^- 0.497 MeV; $T_{1/2} = 6.74$ d) is potentially useful for RIT. Also, ^{177}Lu has useful gamma photons ($\gamma = 0.21$ MeV) for biodistribution and dosimetry studies. Therefore, ^{177}Lu may have the advantages of both ^{131}I and ^{90}Y and may be the most appropriate radionuclide for therapy. Type of radiation and energy associated with some of the prevalent radioisotopes is summarized in the table hereunder:

Table 10: Radionuclides for Therapy:

| Radioisotope | T _½ | Emission | E. Max | Range Max |
|--------------|----------------|----------------|-------------|------------|
| | Hours | | KeV | mm |
| I-131 | 193 | β ⁻ | 610 | 2.9 |
| | | γ | 364 | |
| Lu-177 | 162 | β ⁻ | 498 | 2.0 |
| | | γ | 208 | |
| Y-90 | 64 | β ⁻ | 2250 | 11.0 |
| Bi-213 | 0.76 | α | 8400 | 0.1 |
| | | γ | 440 | |
| At-211 | 7.2 | α | 5870 & 7450 | 0.055-0.08 |
| | | X- | 77-92 | |
| Ac-225 | 240 | α | | |
| | | γ | | |
| Ra-223 | 273.6 | α | 5871 | |
| Th-227 | 449 | α | | |
| | | γ | | |

B. Rationale for Dose–Fractionated regimen:

Pre–clinical models do not always predict toxicity in humans, but based upon the available data, the pre–clinical models combined with animal and human dosimetry data have predicted the toxicity seen with anti–PSMA radioimmunotherapy (i.e. radiolabeled monoclonal antibody) and anti–PSMA radioligand therapy (i.e. radiolabeled peptides).

With radioimmunotherapy, one would predict that the dose–limiting organ is bone marrow, based upon the kinetics / circulation time and exposure of the bone marrow to circulating radiolabeled antibody before it gets into tumors days later. With radioligand therapy, one would predict that there is comparatively very little bone marrow exposure, but there would be more exposure to sites of PSMA expression that are not exposed to (bulky) full–length antibodies. Based upon the available data, this has been born out in human subjects (for radioimmunotherapy) and human patients (for radioligand therapy).

We have conducted a series of overlapping, but sequential phase I and II clinical trials evaluating radiolabeled anti–PSMA monoclonal antibody J591. These studies are summarized immediately below with additional details about dose–fractionation (as requested) following the summary.

- Initial (1st in human) studies of trace–labeled ¹¹¹In–DOTA–J591: demonstrated safety and targeting of J591 in men with mCRPC(32, 89, 90)
- Phase I (single–dose) ⁹⁰Y–J591: demonstrated safety, targeting, and preliminary efficacy in mCRPC(31)
- Phase I (single–dose) ¹⁷⁷Lu–J591: demonstrated safety, targeting, and preliminary efficacy in

mCRPC(28)

- Phase II (single dose) ^{177}Lu -J591: demonstrated efficacy in initial 32 subjects, subsequent analysis with significant dose-response and preliminary biomarkers(91)
- Phase I fractionated-dose ^{177}Lu -J591 dose-escalation study: demonstrated safety, confirming hypothesis that higher cumulative doses could be administered as a split dose(92)
- Phase II (single dose) ^{177}Lu -J591 expansion phase: confirmed efficacy and dose-response with exploratory biomarkers(39)
- Phase I fractionated-dose ^{177}Lu -J591 plus docetaxel: demonstrated the ability to safely combine fractionated ^{177}Lu -J591 with docetaxel 75 mg/m² in men with mCRPC(93)
- Phase I fractionated-dose ^{177}Lu -J591 expansion phase: provided efficacy data, confirmed dose-response, continued analysis of exploratory biomarkers(41)

Taken together, with >250 patients treated on these sequential clinical trials, we have drawn the following conclusions: (i) there is a clear dose-response with more responses and longer survival at higher doses; (ii) there is clear dose-toxicity response in terms of myelosuppression (which is both predictable and reversible); (iii) dose-fractionation allows higher cumulative dose with comparatively less toxicity (including allowing concurrent chemotherapy); and (iv) without pre-selection for PSMA expression, approximately 90% have accurate tumor targeting, though the small minority with low/no PSMA expression by imaging have a lower likelihood of response. While ^{177}Lu -J591 mAb demonstrated significant efficacy, the major limitation in the optimization of therapeutic dose is the hematological toxicity (described in greater detail below).

With a single-dose, 70 mCi/m² of ^{177}Lu -J591 was the MTD, with dose-limiting myelosuppression in those receiving 75 mCi/m² (with 2 of 3 with dose-limiting hematologic toxicity)(28). In the phase II study, this was confirmed with 70 mCi/m² of ^{177}Lu -J591 being tolerable with reversible myelosuppression, but with a significant amount of manageable grade 4 myelosuppression (including approximately 40% platelet transfusion)(39).

In radiotherapy, the anti-tumor response has been thought to be primarily due to induction of apoptosis by radiation(94-97). However, the degree of anti-tumor response following the administration of targeted radionuclides depends on several variables, especially total (cumulative) radiation dose to the tumor, dose-rate and tumor radiosensitivity. Single-agent RIT, although potentially useful for slowing solid tumor growth, has not been effective in controlling aggressive tumors, which often have p53 and other mutations and are less susceptible to apoptosis, the apparent mechanism of cell death from low dose-rate radiation(98). Bone marrow is the dose-limiting organ in RIT in the absence of marrow reconstitution(99, 100). **Dose-fractionation** is a practical strategy to decrease the dose to bone marrow while increasing the cumulative radiation dose to the tumor at an optimal dose-rate(97, 101, 102). The main reason for using dose fractionation is to take advantage of the difference between early-responding and late-responding tissues. The radiation effect on early-responding tissue can be reduced by prolonging the treatment time and dose fractionation. The radiation effect on late-responding tissues will not be changed significantly if the total dose is not changed(97, 102). Preclinical data have shown that dose fractionation or multiple low dose treatments can decrease toxicity while increasing the

efficacy(96, 103, 104). Similarly, there is some clinical evidence that bone marrow toxicity can be reduced with some modest increase in the cumulative maximum tolerated dose(105-107).

Based upon these data, we performed two sequential studies of fractionated dose ^{177}Lu -J591.

As described in in the paragraph above, dose-fractionation of external-beam radiation as well as radioimmunotherapy has been demonstrated to have less toxicity and might be more efficacious by delivering active radiation over a more prolonged period of time to tumor cells. Based upon the half-life of ^{177}Lu -J591 combined with the kinetics of radiolabeled intact monoclonal antibody, we chose a 2-week interval between ^{177}Lu -J591 doses:

i) Phase I trial of fractionated dose ^{177}Lu -J591 in men with metastatic castration-resistant prostate cancer(41, 92)

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 weeks 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 heme toxicities; 19 (57.6%) had Grade 4 heme 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 11: Toxicities data from Phase 2 single dose ¹⁷⁷Lu–J591 Study and Phase 1 fractionated ¹⁷⁷Lu–J591 study:

| | Single Dose | | Fractionated Dose | | |
|---------------------------------------|-------------|-------|-------------------|-----------------|-----------------|
| | 65 | 70 | 70 (35 + 35) | 80 (40 + 40) | 90 (45 + 45) |
| Cumulative Dose (mCi/m ²) | 65 | 70 | 70 (35 + 35) | 80 (40 + 40) | 90 (45 + 45) |
| Platelets Grade 4 | 27% | 56.3% | 40% | 50% | 58.8% |
| Platelet Transfusion | 7% | 41% | 0% | 31.3% | 52.9% |
| Neutropenia Grade 4 | 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:

ii) Phase I trial of fractionated dose ¹⁷⁷Lu–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 ¹⁷⁷Lu. Based upon this theory, a phase I trial of docetaxel and prednisone with escalating doses of fractionated ¹⁷⁷Lu–J591 was initiated. 15 men with median age 69.1 (49.3–80.8) were enrolled. The MTD/RP2D of ¹⁷⁷Lu–J591 was 40 mCi/m²x2 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%.

Aside from the overall rates of dose limiting toxicity, the combined studies have also consistently demonstrated the kinetics of myelosuppression, with nadir neutrophil and platelet counts 28–32 days following the last administered dose of ¹⁷⁷Lu–J591.

With small–molecule radioligand therapy, both the predicted and reported toxicities are much different. One would predict very little myelosuppression, but if it were to occur, it would be

expected to have kinetics similar to (or more quickly than) typical cytotoxic chemotherapy, as the clearance of the myelotoxic drug is quick (minutes to hours rather than days to weeks for antibodies). In fact (with the known caveats of retrospective studies), this is the reported human experience. Essentially no grade 4 neutropenia or thrombocytopenia has been reported within months of exposure to ^{177}Lu -PSMA radioligands (see details below).

While we have published on the long-term follow up with ^{177}Lu -J591 with no cases of attributable permanent myelosuppression and/or myelodysplasia/leukemia, results are unknown with ^{177}Lu -PSMA RLT. The available data with up to 6 months of reported post-treatment follow up are:

- 1) no attributable grade 4 hematologic (or other) toxicity has been reported at any time (the one case of grade 4 thrombocytopenia is reported with a patient with grade 4 platelet count at pre-treatment baseline)
- 2) repeat dosing has been performed in many patients at 8-12 weeks after initial dosing and in all cases, white blood cell count and platelet counts were acceptable prior to subsequent doses (up to 5 doses, with dose #5 approximately 40 weeks after dose #1)

The Table below is a summary of the prior summary anti-PSMA RLT Table which includes the severe leukocyte/neutrophil and platelet count rates following ^{177}Lu -radiolabeled anti-PSMA peptides. In addition to the Table below, a multicenter review of ^{177}Lu -PSMA-617 data of 82 patients receiving a single dose (mean 5.9 GBq) revealed no grade 4 hematologic or non-hematologic toxicity(108).

Table 12: Toxicities data following anti-PSMA radioligand therapy:

| Radioligand Therapy | N | Dose (GBq), Range | Total doses | Grade 4 neutropenia | Grade 4 platelets | Reference |
|---------------------------------|----------|--------------------------|--------------------|----------------------------|--------------------------|--------------------------|
| ¹⁷⁷ Lu-PSMA-617 | 30 | 3.7 – 6 | 1–3 | 0 | 0* | Kratochwil et al 2015 |
| ¹⁷⁷ Lu-PSMA I&T | 56 | 3.6 – 8.7 | 1–5 | 0 | 0 | Baum et al 2016 |
| ¹⁷⁷ Lu-PSMA-617 | 9 | 5.28 – 5.77 | 1 | NR | NR | Hohberg et al 2016 |
| ¹⁷⁷ Lu-DKFZ-PSMA-617 | 5 | 3.4 – 3.9 | 2 | NR | NR | Delker et al 2015 |
| ¹⁷⁷ Lu-DKFZ-PSMA-617 | 10 | 4.1– 6.1 | 1 | 0 | 0 | Ahmadzedeifar et al 2016 |
| ¹⁷⁷ Lu-PSMA-617 | 7 | 5.5 – 7.4 | 1 | NR | NR | Das et al 2016 |
| ¹⁷⁷ Lu-PSMA-617 | 23 | 7.4 | 1 | NR | NR | Demir et al 2016 |
| ¹⁷⁷ Lu-PSMA-617 | 28 | 6 | 1–2 | 0 | 0 | Rahbar et al 2016 |
| ¹⁷⁷ Lu-PSMA-617 | 31 | 1.1– 5.5 | 1–4 | 0 | 0 | Yadav et al 2016 |
| ¹⁷⁷ Lu-PSMA I&T | 2 | 5.7 – 8 | 1 | 0 | 0 | Weineisen et al 2015 |
| ¹⁷⁷ Lu-PSMA I&T | 22 | 3.4 – 7.4 | 1–4 | 0 | 0 | Heck et al 2016 |
| ¹⁷⁷ Lu-PSMA-617 | 43 | 6 | 1–4 | 0 | 0 | Zimbelmann et al 2016 |
| ¹⁷⁷ Lu-PSMA-617 | 5 | 5.8 – 7.4 | 1 | 0 | 0 | Chakraborty et al 2016 |

*One patient had pre-treatment baseline platelet count of grade 4
NR = not reported

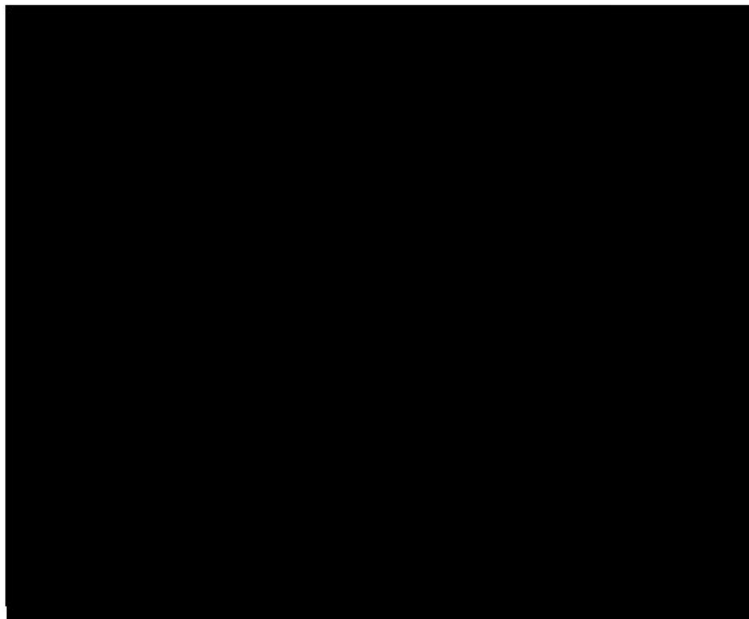
A dose fractionated regimen provides the theoretic benefit of higher cumulative radiation dose to the target tissue/organ while limiting toxicities secondary to non-specific binding of the radiolabeled ligand. In addition to the toxicity data provide above, our group has consistently seen evidence of dose-response (see Table below), justifying a dose-escalation study.

Table 13: Dose response from the Phase-I fractionated ¹⁷⁷Lu-J591 study:

| Group | N | Any PSA decline | ≥ 30% PSA decline | > 50% PSA decline | Median OS mo [95% CI]* |
|--------------|----|-----------------|-------------------|-------------------|------------------------|
| Total | 49 | 55.1% | 32.7% | 16.3% | 22.9 [16.2, 29.7] |
| Low doses | 16 | 37.5% | 12.5% | 6.3% | 14.6 [9.9, 19.4] |
| RP2D | 33 | 66.7% | 42.4% | 21.2% | 27.7 [15.8, 39.6] |
| 40 x2 cohort | 16 | 50.0% | 25.0% | 12.5% | 19.6 [9.1, 30.2] |
| 45 x2 cohort | 17 | 87.5% | 58.8% | 29.4% | 48.3 [16.0, 80.6] |

C. Rationale for combination regimen:

PSMA represents an example of a cellular target that can be co-targeted by 2 different agents that bind to different sites on the PSMA molecule and where each agent has significantly different properties. The crystal structure of PSMA has previously been determined. The binding site of the J591 antibody has been mapped to the apical domain of PSMA, furthest away from the cell membrane (see figure below).



In this exemplary case, the antibody and small molecule (enzymatic inhibitor) have very different properties as shown in the table below.

Table 14: Differential characteristics of anti-PSMA antibody (J591) and anti-PSMA peptides (PSMA-617):

| Characteristic | Antibody | Peptide |
|--------------------------------|--------------------------|---|
| Size (Molecular weight) | 150 kd | 1 kd |
| Kinetics (blood half-life) | 3-7 days | 3-7 hours |
| Bio-distribution | Tumor , Blood | Tumor , Salivary glands, Kidneys |
| Side effects | Transient ↓ blood counts | Dry mouth/eyes; ↓ kidney function |
| Route of elimination from body | Via liver | Via urinary tract |

As a result of the differing properties, the predicted side effects caused by the 2 targeting agents and their cytotoxic payloads are virtually mutually exclusive and the actual dose-limiting toxicity of the individual agents in humans is also consistent. This allows co-administration of the 2 targeting agents with their payloads to provide additive payload dose to the tumor without increasing side effects.

Our group has recently conducted both in-vitro and in-vivo experiments to confirm:

1. [REDACTED]



[Redacted text block]

[REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

2. [REDACTED]
[REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Summary:

Taken together, these data show that co-administering 2 agents that bind different sites of a target molecule (e.g., a cell surface receptor molecule) present on a population of cells results in additive binding of those agents. Where the target molecule/receptor is internalized, additive amounts of the 2 agents will, therefore, be internalized. Furthermore, if the 2 targeting agents have different properties (e.g., molecular mass, charge, hydrophilicity/hydrophobicity, pharmacokinetics, bio-distribution, etc.) such that their respective side effects differ, and are non-overlapping, the 2 agents can be co-administered to result in additive binding/uptake by the targeted cells without causing any added toxicity. It is clear across our prior PSMA-targeted radionuclide therapy studies that higher administered doses lead to more responses and longer survival, but also increased toxicity. As a corollary, the dose of each agent can be modestly reduced (by 5–50%) below its

respective maximum tolerated dose whereby the co-administration of the 2 targeting agents still provides an additive dose to the target cells but can substantially or completely reduce the toxicity experienced by the subject.

2.8 Risk/Benefit Assessment

This is a phase I clinical trial with the primary endpoints to find DLT and MTD. Based on the prior clinical studies using ^{177}Lu -J591 by our group the dose-limiting organs/tissue is expected to be red marrow. The expected toxicities can be leukopenia, thrombocytopenia, anemia, fatigue, renal failure, and transaminitis. All these toxicities have been transient and reversible. mCRPC patients treated with RIT using ^{177}Lu -J591 have shown improved survival compared to historical controls.

Based on findings by retrospective publications, the most common side effects and/or toxicities reported for ^{177}Lu -PSMA-617 or ^{68}Ga -PSMA-HBED-CC are fatigue, renal failure, xerostomia, leukopenia, anemia, thrombocytopenia (so far none of the published studies have reported any significant hematological toxicities) and transaminitis. All of these toxicities are reportedly mild and transient. mCRPC patients treated with RLT have higher response rates than any available standard drug in the refractory setting by historical standards.

2.9 Correlative Studies Background

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

We will incorporate tissue-based and circulating tumor DNA (ctDNA) approaches to assess prostate cancer pathway changes including AR signaling pathway activation (mRNA) and genomic alterations including DNA repair pathways and will correlate with PSMA heterogeneity on imaging. We will evaluate the association of molecular biomarkers with imaging and treatment response. Mechanisms of resistance will be assessed in a subset of patients enrolled at WCM through integrative genome-wide analysis of pre-treatment and progression tumor biopsies. At the end of this Aim, we will have defined the molecular landscape of CRPC patients enrolled on prospective trials of PSMA-targeted radionuclide therapy and will have identified biomarkers associated with outcome to be evaluated in a larger study to determine whether they are predictive or prognostic.

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 PSMA-targeted radionuclide therapy. 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 ¹⁷⁷Lu. 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 prostate cancer specific targeted platform covering DNA (for mutation and copy number) of 74 genes/regions commonly aberrant in prostate cancer and 42 control genes. Prostate cancer genes including AR, SPOP, CHD1, DNA repair, ERG fusion, and other CRPC-relevant alterations. We will also collect a single-sample of blood for germline analysis of DNA repair defects. 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. The panel is also being used for other prospective therapeutic trials which will allow for cross-comparison of data.

Gene expression of androgen signaling genes and other relevant pathways will be assessed using a custom panel Nanostring platform developed and validated by Dr. Beltran's lab. This panel includes 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. As discussed above, we will describe findings from this exploratory endpoint in each study with the plan to analyze identified biomarkers to determine their association with efficacy and toxicity across studies, in particular at doses found to have efficacy.

ii. CTC Count:

Circulating tumor cell (CTC) counts via the FDA-cleared, standard of care 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 (109). 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 (110)

iii. Immune Assays:

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. In prostate cancer, humoral immune response has already been documented with immunotherapy. *We hypothesize that prostate cancer is immunogenic following PSMA-targeted radiation, and that the serological repertoire in patients may be useful as a pharmacodynamic, prognostic, or even predictive tool.*

We propose to test the hypothesis that radiolabeled anti-PSMA treatment of advanced prostate tumors leads to induction or increase in immune responses to locally expressed antigens by assessing serological changes pre- and post-treatment. Indeed, one surrogate to test whether these tumors become more immunogenic is to measure naturally occurring antibodies in the serum or plasma of patients. Autologous serotyping, i.e., serum antibody recognizing tumor-derived antigens, have resulted in a growing list of human cancer antigens that are immunogenic in the host of origin. With regard to cancer antigens recognized by the humoral immune system, over 2700 sequences have been identified by SEREX analysis of sera from patients with a wide range of cancers; these antigens include mutational, overexpressed, oncogenic viral, differentiation, and cancer-testis (CT) antigens. These can be rapidly tested in high-throughput ELISA using recombinant antigens (Grand Serology). In some cases, such as the cancer/testis antigen NY-ESO-1, serum antibodies are associated with spontaneous T cell responses measurable from peripheral blood. It should be noted that most of the antigens defined as targets of autoantibodies are intracellular, rather than surface antigens, and reflect the capacity of the immune system to detect abnormalities associated with neoplasia. The prevalent hypothesis is that antibodies are generated as antigens are released from dying tumor cells, either because of spontaneous necrosis or because of external treatments. The presence of antibodies to intracellular targets can be used as a marker of tumor existence or progression, but can also have a functional role to help generating T cell responses through the formation of immune complexes with their cognate antigen. Today, protein microarrays allow for the detection of specific serum antibodies against a very large number of targets simultaneously. Arrays can be used to determine patterns of antigens recognized by autoantibodies during the course of diseases. These tools are also useful to measure changes in antibody responses with treatment such as immunotherapies. The opportunity to define “seromic” changes in these prostate cancers, i.e., the scope of antigens recognized with increased specificity and frequency by post-treatment patient sera vs. baseline, is now within reach, provided adequate analyses of the vast amount of data generated by these microarray can be properly interpreted.

Grand serology: A series of known tumor antigens will be assessed in a hypothesis-driven manner for their capacity to elicit autoantibodies in advanced prostate cancer patients, at baseline and after treatment with PSMA-TRT. Using ELISA as previously described, we propose to test a series of 20-25 tumor antigens, including mutational, stem-cell, endogenous retroviral, and cancer-testis antigens such as TP53, NY-ESO-1, GAG-HERV-K, SOX2, PRAME, WT1, MAGE-A3, SSSX2, CT45, etc., most of which already have demonstrated spontaneous immunogenicity various solid tumors, including advanced prostate cancer. Briefly, sera will be

tested in 4x serial dilutions, starting from 1/100, for IgG reactivity against full-length E. coli- or yeast-produced proteins of tumor and control antigens to assess specificity. Positive and negative control sera with known reactivity will be used on each plate to validate the assays. Reciprocal titers will be extrapolated and considered significant if >100 . In samples with multiple serum collection time points available, significant changes in humoral response will be defined as antigen-specific antibody going from undetectable (titer <100) to detectable (titer >100), or with titers at least 4-fold different between time points. For antigens showing humoral reactivity, mapping of linear epitopes may be assessed using overlapping peptide series covering the sequence of the antigen, to assess polyclonality and potential spreading over time. In a subset of patients, other immunoglobulin isotype or subclass usage may be assessed as well. We propose that systemic tumor antigen-specific antibody responses in plasma or serum may be useful as a marker of immunological response to radiolabeled anti-PSMA treatment in advanced prostate cancer patients, and that increases or induction in immune responses will occur with treatment in comparison to baseline. These experiments are expected to shed light on both mechanisms of action of the drug, including occurrence of antigen spreading, and on potential predictive biomarkers.

Seromics: While Grand Serology offers a high-throughput way to quickly assess changes of antibody specificity in serum or plasma sample to known tumor antigens, it is limited by the small number of tumor targets identified to date, especially in prostate cancer. To circumvent this limitation, an alternative is to use a broader array of self-antigens instead as potential targets of immune responses generated by immunotherapy.

Seromics is an exploratory, non-biased, hypothesis-generating platform that allows the testing of thousands of human proteins simultaneously as potential targets of autoantibodies from patient serum or plasma. The method only requires a few microliters of material, and may be customized for applications other than IgG antibody detection. The seromic platform can be used to define biomarkers or sets of antigens present at baseline in specific patient populations, and that could be used as prognostic or predictive markers. It has been previously described that prostate cancer spontaneously elicits humoral responses to a small subset of autoantigens that are not immunogenic in healthy donors. Accordingly, seromics is suited to comprehensively look for serum antibody changes in prostate cancer patients at the antigen-specific level between consecutive time points, before and after treatment with radiolabeled anti-PSMA therapy. We plan to use either ProtoArrays (Invitrogen) or HuProt (CDI), protein microarrays spotted with 9000 to 20000 cancer- and non-cancer associated proteins and controls, to define serum autoantibody changes in a subset of proposed treated patients (pre-post) with favorable outcomes, and compare them to changes in age-matched patients with no response.

Immunogenic cell death: we hypothesize that the PSMA TRT will lead to generation of an immune response that will induce immunogenic cell death.

High mobility group box 1 (HMGB1) is an important mediator of immunogenic cell death, and it has been shown that it is released by dying tumor cells following radiation. HMGB1 may promote dendritic cell activation and migration and antigen presentation; following certain anti-neoplastic therapy (such as radiation) higher levels of HMGB1 have been associated with improved

outcomes. We will assess HMBG1 via ELISA in serum obtained pre-and post-treatment with PSMA-TRT and analyze for associations with response, progression-free survival, and overall survival outcomes. We have stored serum (previously collected for HAHA assessment) from prior studies with long-term outcome data and continue to prospectively collect serum from patients being enrolled in current PSMA-TRT studies.

Among the several markers of immunogenic cell death, calreticulin is one of the most investigated. Calreticulin exposure on tumor cell surface is widely accepted as an “eat-me” signal, which is required for phagocytosis of dying tumor cells. Mainly an endoplasmic reticulum (ER)-associated chaperone protein, CTR translocation to the plasma membrane is triggered by reactive oxygen species (ROS)-induced ER stress, dictating the immunogenic cell death. We hypothesize that calreticulin tumor cell expression and cellular localization is associated with tumor response to the immunogenic response-inducing PSMA TRT. Unfortunately, the availability of pre- and post-treatment tumor tissue is often a logistical problem in patients with prostate cancer. We expect to have a subset with pre- and post-treatment tumor tissue available and we will assess membrane expression of CRT in that subset.

[REDACTED]

[REDACTED]

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/GnRH 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 $\geq 2,000$ cells/mm³
 - Hemoglobin ≥ 9 g/dL
 - Platelet count $\geq 150,000 \times 10^9/\mu\text{L}$
 - Serum creatinine ≤ 1.5 x upper limit of normal (ULN) or calculated creatinine clearance ≥ 60 mL/min/1.73 m² by Cockcroft–Gault

- Serum total bilirubin $\leq 1.5 \times \text{ULN}$ (unless due to Gilbert's syndrome in which case direct bilirubin must be normal)
 - Serum AST and ALT $\leq 1.5 \times \text{ULN}$ in the absence of liver metastases; $< 3 \times \text{ULN}$ 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 Treatment visit #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 Treatment visit # 1 (Day 1) or current enrollment in investigational oncology drug or device study
3. Prior systemic beta-emitting bone-seeking radioisotopes
4. Known active brain metastases or leptomeningeal disease
5. History of deep vein thrombosis and/or pulmonary embolus within 1 month of Treatment visit #1
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 Treatment visit #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 combination of ¹⁷⁷Lu-J591 and ¹⁷⁷Lu-PSMA-617 in a two-week dose-fractionation regimen. ¹⁷⁷Lu-J591 will be given at a moderate dose previously demonstrated to be safe x2 infusions two weeks apart. For ¹⁷⁷Lu-PSMA-617 the dose escalation will start at 3.7 GBq (100 mCi) and escalate in increments of 1.85 GBq (50 mCi) for each dose to a planned maximum of 9.25 GBq (250 mCi) x2 doses, 2 weeks apart. Should there be unacceptable toxicity at the initial dose level, we will de-escalate to dose level -1 (1.85 GBq / 50 mCi per dose). After the phase I study has established a MTD, the Phase II, single-arm trial will start.

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 ⁶⁸Ga-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).

Subjects will have Lutetium-177 Planar/SPECT Imaging on Day 8 (±1 day) after the first dose of ¹⁷⁷Lu-J591 + ¹⁷⁷Lu-PSMA-617. Optimal images will be performed on selected consenting subjects between the initial treatment visit #1 on Day 1 and Day 4 and prior to treatment visit #2 on D15 ±1. Subjects will be closely monitored for AEs (weekly x2 weeks, then every 2 weeks for one month, at 8 and 12 weeks, and then every 4 weeks for next 3 months).

Upon completion of investigational treatment with dose-fractionation regimen of the combination of ¹⁷⁷Lu-J591 + ¹⁷⁷Lu-PSMA-617, subjects will undergo ⁶⁸Ga-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 14: Phase I dose-escalation plan for combination of ¹⁷⁷Lu-J591 + ¹⁷⁷Lu-PSMA-617:

| ¹⁷⁷Lu–J591 + ¹⁷⁷Lu–PSMA–617: Dose escalation (per dose) | | |
|--|----------------------------------|----------------------------------|
| | ¹⁷⁷Lu–J591* | ¹⁷⁷Lu–PSMA–617 |
| | Dose: GBq (mCi) / m ² | Dose: GBq (mCi) |
| Cohort | | |
| -1 | 2.22 (60) | 1.85 (50) |
| 1 | 2.22 (60) | 3.7 (100) |
| 1a | 1.35 (36.5) | 3.7 (100) |
| 2 | 1.35 (36.5) | 5.55 (150) |
| 3 | 1.35 (36.5) | 7.4 (200) |
| 4 | 1.35 (36.5) | 9.25 (250) |

*Note: BSA will be capped at 2 for ¹⁷⁷Lu-J591 dosing.

Rationale for dosing amendment:

Prior studies of ¹⁷⁷Lu- and ⁹⁰Y-J591 have utilized individualize dosing per body surface area (BSA). As a single agent, ¹⁷⁷Lu-J591 when delivered in a 2-dose fractionated regimen is suggested to be dosed at 45 mCi/m² x2 (or 40 mCi/m² x2 with less toxicity and less efficacy). The dose in combination with docetaxel is 40 mCi/m² x2). In the initial version of the protocol, an estimate for fixed dosing based upon prior safe dose levels and average BSA was utilized. One subject in the initial cohort had prolonged myelosuppression and on analysis, had a low BSA. Therefore based upon the wealth of prior safety data with BSA dosing and the wish to primarily assess one rather than 2 dosing variables, the dosing amendment will revert to BSA dosing. In addition, we will cap BSA at 2 m² with the intent of minimizing myelosuppression from the RIT.

4.2 Phase I:

The Phase I portion will use a 3+3 dose-escalation study design, with the planned initial and subsequent cohorts described in Table 14. This study design is well-described and accepted by the clinical and scientific community, so we will not provide complete details here. 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. The MTD 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: | .05 | .10 | .20 | .30 | .40 | .50 | .60 |
| Probability of Escalation: | .97 | .91 | .71 | .49 | .31 | .17 | .08 |

As there is a reasonably large pool of short- and long-term experience with PSMA-directed ¹⁷⁷Lu, a phase II trial using the MTD to will be launched to obtain preliminary efficacy data.

4.3 Phase II:

We will enroll up to 27 subjects in the Phase II trial utilizing a Simon two-stage design (including the 3-6 patients used for establishing the MTD). For the phase II portion, we will define evaluable patients as those who met eligibility requirements, have initiated therapy, and were not removed from the study for non-compliance or withdrawal. Sample size recommendations for the two-stage design are determined according to Simons two-stage minimax design. The first stage will accrue a total of 16 patients (including those who were treated at the MTD in the phase I portion), if 6 or fewer of the first 16 evaluable patients do not experience a 30% decline in PSA, the study will be terminated and declared to have a negative result. If 7 or more patients out of the first 16 evaluable patients experience a 30% decline in PSA, 9 more patients will be accrued in Stage 2 (total sample size of 27). The new regimen will be declared to have activity and be worthy of further testing if 13 or more patients experience a 30% PSA decline among the 27 patients who are part of the phase II trial.

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 15** and described in detail in **Section 6.1**.

6.1 Schedule of Evaluations

Table 15: Schedule of trial events:

| | Screening | Treatment Visit 1 | Imaging visit | Treatment Visit 2 | F/U Visit 1 | F/U Visit 2 | F/U Visit 3 | F/U Visit 4 | F/U Visit 5 | Scan / Efficacy Visit | Short-Term F/U ^k | Long-Term F/U |
|--|-----------|-------------------|---------------|-------------------|-------------|-------------|-------------|-------------|-------------|-----------------------|-----------------------------|----------------|
| Informed Consent | x | | | | | | | | | | | |
| Demographics | x | | | | | | | | | | | |
| Medical History | x | x | x | x | x | x | | x | | x | x | |
| Physical Exam | x | x | x | x | x | x | | x | | x | x | |
| Performance Status | x | x | x | x | x | x | | x | | x | x | |
| Vital Signs ^a | x | x | x | x | x | x | | x | | x | x | |
| PRO ^l | x | x | x | x | x | x | | x | | x | x ^l | |
| CBC with diff, plts ^b | x | x | x | x ^c | x | x | x | x | x | x | x | |
| Serum Chemistry ^d | x | x | x | x | x | x | x | x | x | x | x | |
| 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 | | |
| CTC for Research ^f | x | x ^f | | | | | | | | x | | |
| Cell-Free DNA Research sample ^g | x | x ^g | | | | | | | | x | | |
| Immune research sample ^m | x | x ^m | | | | | | | | x | | |
| ⁶⁸ Ga-PSMA infusion & PET CT | x | | | | | | | | | x | | |
| Radiographic evaluation ^h | x | | | | | | | | | x | x ^h | x ^h |
| ¹⁷⁷ Lu-J591 infusion | | x | | x | | | | | | | | |
| ¹⁷⁷ Lu-PSMA-617 infusion | | x | | x | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|----------------|----------------|---|---|--|---|--|---|---|---|
| ¹⁷⁷ Lu-Planar/SPECT imaging ⁱ | | | x ⁱ | x ⁱ | | | | | | | | |
| Archival Tissue | x | | | | | | | | | | | |
| Adverse Event Monitoring | | x | x | x | x | x | | x | | x | x | |
| Concurrent Medications | x | x | x | x | x | x | | x | | x | x | |
| Survival Assessment ^j | | | | | | | | | | | | x |

- a: Vital signs will include height and weight during screening and at least weight thereafter
- b: Any subject with grade >2 neutropenia or thrombocytopenia at any time point will be followed at least weekly until resolution to grade 1
- c: Any subject with grade ≥2 neutropenia or thrombocytopenia at Treatment 2 will initially not be eligible to receive the second ¹⁷⁷Lu-J591 / ¹⁷⁷Lu-PSMA-617 infusions. The dose will be held until the toxicity has resolved to grade 1 for a maximum of 2 weeks. If the toxicity has not resolved after 2 weeks, the dose will be discontinued.
- 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: CTC collection for research purposes may be performed during screening or prior to treatment C1D1: two 2.3% sodium citrate “light blue top” tubes
- g: Cell-Free DNA BCT[®] may be obtained during screening or prior to treatment C1D1 and will be whole blood collection in a tube intended for collection, stabilization and transportation of cell-free plasma DNA. 10 mL tube will be used for collecting whole blood for research purposes
- 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: Planar/SPECT imaging one week after the ¹⁷⁷Lu-PSMA-617 1st dose infusion in all subjects. Optional imaging will be performed on consenting subjects between D1-D4 and/or prior to treatment #2 on D15.
- j: Survival assessment and relevant medical history to be collected until death
- k: Short-term follow up to be completed q4 weeks following the scan visit until 6 months from the 1st treatment visit. Subjects who are progression-free at 6 months will continue with q4-week labs and q12-week standard imaging per guidelines (as in h above).
- l: PRO = patient reported outcomes = BPI-SF and FACT-P; to be performed at time points specified during treatment phase, then q12 weeks (at imaging time points) during short-term follow up until radiographic progression
- m: serum for immune assays; 1 sample to be collected at any time during screening prior to treatment #1, then again during scan/efficacy visit

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.

- 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
- Brief Pain Inventory
- 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 C1D1, do not need to repeat if has known result within 1 month of C1D1)
- CTC for Research (may be collected prior to treatment C1D1)
- Cell-Free DNA BCT[®] Research blood sample (may be collected prior to treatment C1D1)
- Immune research blood sample (may be collected 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)
- Single intravenous dose of ⁶⁸Ga-PSMA-HBED-CC (5±2mCi or 185±74MBq) at least 1 week prior to ¹⁷⁷Lu-PSMA-617 infusion
- ⁶⁸Ga-PSMA-HBED-CC PET/CT scan to confirm the PSMA expression at the tumor site(s). PET/CT scans will be obtained 1 to 3 hours after the infusion of ⁶⁸Ga-PSMA-HBED-CC
- Archival tissue for PSMA expression and genomic studies to identify DNA damage

Screening (except ^{68}Ga -PSMA-HBED-CC PET/CT scan) and visit #1 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.

6.1.1.1. Re-screening

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

6.1.2. Treatment Phase

The treatment and early monitoring phase comprises of 8 visits spanning over approximately 12 weeks.

Absolute neutrophil counts, platelet counts, bilirubin, transaminases, and serum creatinine must be performed with results available and within range of eligibility criteria within 1 week prior to treatment initiation on C1D1.

Details for each visit are listed below:

6.1.2.1 ^{177}Lu -J591 and ^{177}Lu -PSMA-617 infusion (Treatment 1 Day 1) and (Treatment 2 Day 15 \pm 1 day)

The following procedures must be completed on the day of treatment:

- 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
- Intravenous dose of ^{177}Lu -PSMA-617 (Dose based on the Cohort in which the subject is enrolled) and ^{177}Lu -J591
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures
- Planar/SPECT imaging (on D15 prior to treatment in consenting subjects)

As stated above, Absolute neutrophil counts, platelet counts, bilirubin, transaminases, and renal function assessment must be available within 1 week prior to treatment on D1 and meet eligibility criteria.

For treatment #2 (D15), grade ≥ 2 thrombocytopenia or neutropenia within 24 hours of the second dose of ^{177}Lu -PSMA products (Visit 3) requires a subject's treatment to be held until the toxicity has resolved to least grade 1. If the toxicity has not resolved after 2 weeks of monitoring, the subject's ^{177}Lu -PSMA product treatment will be permanently discontinued and will be termed dose-limiting toxicity

6.1.2.2 Imaging visit (Day 8 \pm 1) [optional D1, D4, D15 in consenting subjects]

- Planar/SPECT imaging
- 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)
- Adverse event evaluation
- Concomitant medications

6.1.2.2 Follow-up visits: Follow up Visit 1 (Day 22 \pm 3), Follow up Visit 2 (Day 29 \pm 3), Follow up Visit 4 (Day 57 \pm 3)

- 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 (Follow up visits 2 & 4)
- LDH (Follow up visits 2 & 4)
- Adverse event evaluation
- Concomitant medications

6.1.2.3 Follow-up lab visits: Follow up Visit 3 (Day 43 \pm 3); Follow up Visit 5 (Day 71 \pm 3)

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

Lab visits may be performed at any CLIA certified lab

6.1.3. Efficacy Evaluation (scan) Visit (Day 85 ± 7; note that these study procedures are not expected to occur on a single day)

The following procedures must be completed during the efficacy visit(s):

- 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
- CTC for research
- Cell-Free DNA BCT[®] Research blood sample
- Immune research blood sample
- Same standard radiographic imaging modality as baseline (CT/MRI and bone scan +/- CXR)
- Single intravenous dose of ⁶⁸Ga-PSMA-HBED-CC (5±2mCi or 185±74MBq)
- PET/CT scan will be obtained between 1 and 3 hours post-injection of ⁶⁸Ga-PSMA-HBED-CC
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures

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

Unless subjects have radiographic progression, withdraw from study, or initiate new treatment, the following procedures must be completed every 4 weeks (+/- 7 days) after the initial efficacy/scan visit:

- 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

These q4-week lab/clinic 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. Visits associated with repeat CT and bone scan (q12 weeks until radiographic progression) should be performed at the WCM investigative site.

6.1.5 Long Term Follow Up

Following cancer progression, survival assessment and relevant medical history to be collected until death. Information may be collected from external providers. Information will be updated at least every 6 months following disease 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. ¹⁷⁷Lu–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.

The DOTA–J591 antibody molecule is labeled with radiometal ¹⁷⁷Lu in 1.0 M ammonium acetate buffer at pH 7.0. The radiolabeled J591 complex is then purified using size–exclusion column chromatography. To avoid the effects of autoradiolysis on the antibody, reaction time is minimized and the radiolabeled antibody preparations are formulated in phosphate buffered saline containing not more than 1% Human Serum Albumin (HSA). All reagents used in the conjugation and purification of J591 are made with pyrogen–free water. Radiolabeled J591 is periodically tested for sterility and pyrogenicity.

Each patient dose will be supplied to the study site on the day of or day before treatment by the central radiopharmacy. The J591 mAb is a deimmunized monoclonal antibody directed at the

extracellular domain of human PSMA. For additional details, see Section 1. Radiolabeled antibody preparations are formulated in phosphate buffered saline containing not more than 1% Human Serum Albumin (HSA). In order to radiolabel J591 with β^- emitting radionuclides, the antibody molecule is first conjugated to a macrocyclic chelating agent, 1,4,7,10-tetraazacyclododecane- N,N',N'',N''' -tetraacetic acid (DOTA) by direct coupling of one of the four carboxylic acid groups of DOTA to the primary amines present in the protein structure. The radiolabeling of J591 with ^{177}Lu is achieved by adding the radionuclide to the ammonium acetate buffered J591. To avoid the effects of autoradiolysis on the antibody, reaction time is minimized and the reaction mixture is purified with a size exclusion column prior to administration. All reagents used in the conjugation and purification of J591 are made from pyrogen-free water. Radiolabeled J591 is tested periodically for sterility and pyrogenicity. 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 (^{177}Lu -J591, IND# 11613).

B. ^{177}Lu -PSMA-617 and ^{68}Ga -PSMA-HBED-CC

ABX Advanced Biochemical Compounds, Radeberg, Germany produces both peptides/ligands, PSMA-617 and PSMA-HBED-CC, with PSMA-617 licensed to Endocyte (West Lafayette, LA). Upon purchase and shipment to Weill Cornell Medicine, they will be labeled with either ^{177}Lu or ^{68}Ga with final product being ^{177}Lu -PSMA-617 and ^{68}Ga -PSMA-HBED-CC, respectively. 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 (^{177}Lu -PSMA-617, IND # 131966 and ^{68}Ga -PSMA-HBED-CC, IND# 124495).

6.2.3 Route of Administration

^{177}Lu -J591, ^{177}Lu -PSMA-617 and ^{68}Ga -PSMA-HBED-CC will be administered intravenously.

6.2.4 Dose levels for ^{177}Lu -J591 + ^{177}Lu -PSMA-617

Subject(s) enrollment will be done as 3+3 study design at each dose level. Initially at least 3 subject(s) will be enrolled in Cohort 1 and will receive ^{177}Lu -PSMA-617 (2 GBq or 54 mCi x2 doses 2 weeks apart) with dose escalation as described above in **Section 4.1 (Table 14)**.

Simultaneously, all subjects will receive in combination a fixed dose of ^{177}Lu -J591 (1.35 GBq/m² or 36.5 mCi/m² capped at BSA of 2) x2 doses, 2 weeks apart. The enrollment ceiling of the dose escalation portion of the study is up to 30 treated study participants (up to five groups, up to 6 at each dose level). Each patient would receive two doses of both investigational agents (^{177}Lu -J591 and ^{177}Lu -PSMA-617) in combination 2 weeks apart. The total dose received by a subject will vary from **4 GBq/m² (108 mCi/m²) to 20 GBq/m² (540 mCi/m²)** for ^{177}Lu -PSMA-617. The total dose received by a subject for ^{177}Lu -J591 will be **2.7 GBq/m² (73 mCi/m²)**, with BSA capped at 2.

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

All subjected enrolled will receive $5 \pm 2\text{mCi}$ or $185 \pm 74\text{MBq}$ 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 is recommended prior to J591 dosing. Individual subjects may receive pre-medication at the discretion of their treating physician provided that they are not prohibited medications per study. The recommended pre-medication for ^{177}Lu -J591 is a H1 blocker (recommended 25-50 mg diphenhydramine) and acetaminophen (recommended 325-650 mg). No pre-medications will be administered for ^{68}Ga -PSMA-HBED-CC.

6.2.7 Study drug administration

Intravenous access 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 ^{177}Lu -J591, ^{177}Lu -PSMA-617 or ^{68}Ga -PSMA-HBED-CC. ^{177}Lu -J591 will be infused at a rate of no faster than 5 mg/minute. ^{177}Lu -PSMA-617 and ^{68}Ga -PSMA-HBED-CC may be administered as slow IV push.

6.2.8. Monitoring Vital signs pre/post ^{177}Lu -J591 + ^{177}Lu -PSMA-617 and ^{68}Ga -PSMA-HBED-CC

The infusion of ^{177}Lu -J591, ^{177}Lu -PSMA-617 and ^{68}Ga -PSMA-HBED-CC 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) [additionally, at 90 ± 10 minutes and 120 ± 10 minutes for ^{177}Lu -J591 + ^{177}Lu -PSMA-617] after the infusion. If any subject has any adverse reaction at 60 minutes for ^{68}Ga -PSMA-HBED-CC and at 120 minutes for ^{177}Lu -J591 + ^{177}Lu -PSMA-617, they will stay longer until it is resolved.

6.2.9. Imaging Plan

A. ^{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 (efficacy/scan 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.

B. Lutetium-177 Planar/SPECT imaging

Patient preparation should be according to the policies and procedures of the local imaging site. 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 ^{177}Lu -PSMA-617 planar/SPECT images will be provided in study specific documentation by the study chair or the co-investigators from the Division of Nuclear Medicine. Planar/SPECT images should be obtained during the Imaging Visit (Day 8 ± 1) on all subjects after the ^{177}Lu -J591 + ^{177}Lu -PSMA-617 infusion. Image acquisition will be from vertex of skull to mid thighs. Optional images will be performed on selected consenting subjects between initial Treatment Visit (between D1 - D4) and prior to Treatment Visit 2 (Day 15 ± 1).

6.2.10 Managing toxicity

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

- Grade ≥ 2 thrombocytopenia or neutropenia within 24 hours of the second dose of ^{177}Lu -J591 + ^{177}Lu -PSMA-617 (Treatment Visit #2) requires a subject's treatment to be held until the toxicity has resolved to least grade 1. If the toxicity has not resolved after 2 weeks of monitoring, the subject's ^{177}Lu -J591 + ^{177}Lu -PSMA-617 treatment will be permanently discontinued and will be termed dose-limiting toxicity.

Dose-limiting toxicity (DLT) is defined as:

- Grade 4 hematologic toxicity that is deemed at least possibly related to ^{177}Lu -J591 and/or ^{177}Lu -PSMA-617 will be termed dose-limiting toxicity
- Any grade > 2 non-hematologic toxicity deemed to be at least possibly related to ^{177}Lu -J591 and/or ^{177}Lu -PSMA-617 will require a subject's ^{177}Lu -J591 + ^{177}Lu -PSMA-617 treatment to be permanently discontinued and will be termed dose-limiting toxicity.
 - A 1-grade increase in a pre-existing from grade 2 to grade 3 will only be considered a DLT if probably or definitely related to study drug

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 ^{177}Lu -J591, ^{177}Lu -PSMA-617 or ^{68}Ga -PSMA-HBED-CC as judged by the investigator (see note above re: 1-grade increases in which case only probably/definitely related toxicities will be deemed DLT). 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 ^{177}Lu -J591 or ^{177}Lu -PSMA-617
- Previous anaphylactic reaction to any J591 product or PSMA peptides/ligands

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 ^{177}Lu -J591 + ^{177}Lu -PSMA-617 x 2 doses, 2-weeks apart 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. ¹⁷⁷Lu–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.

The DOTA–J591 antibody molecule is labeled with radiometal ¹⁷⁷Lu in 1.0 M ammonium acetate buffer at pH 7.0. The radiolabeled J591 complex is then purified using size–exclusion column chromatography. To avoid the effects of autoradiolysis on the antibody, reaction time is minimized and the radiolabeled antibody preparations are formulated in phosphate buffered saline containing not more than 1% Human Serum Albumin (HSA). All reagents used in the conjugation and purification of J591 are made with pyrogen–free water. Radiolabeled J591 is periodically tested for sterility and pyrogenicity.

Each patient dose will be supplied to the study site on the day of or day before treatment by the central radiopharmacy. The J591 mAb is a deimmunized monoclonal antibody directed at the extracellular domain of human PSMA. For additional details, see Section 1. Radiolabeled antibody preparations are formulated in phosphate buffered saline containing not more than 1% Human Serum Albumin (HSA). In order to radiolabel J591 with β^- emitting radionuclides, the antibody molecule is first conjugated to a macrocyclic chelating agent, 1,4,7,10–tetraazacyclododecane–N,N',N'',N'''–tetraacetic acid (DOTA) by direct coupling of one of the four carboxylic acid groups of DOTA to the primary amines present in the protein structure. The radiolabeling of J591 with ¹⁷⁷Lu is achieved by adding the radionuclide to the ammonium acetate buffered J591. To avoid the effects of autoradiolysis on the antibody, reaction time is minimized and the reaction mixture is purified with a size exclusion column prior to administration. All reagents used in the conjugation and purification of J591 are made from pyrogen–free water. Radiolabeled J591 is tested periodically for sterility and pyrogenicity. 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 (¹⁷⁷Lu–J591, IND# **11613**).

B. ^{177}Lu -PSMA-617 and ^{68}Ga -PSMA-HBED-CC:

PSMA-617 will be obtained from Endocyte (West Lafayette, LA) and PSMA-HBED-CC will be obtained from ABX Advanced Biochemical Compounds, Radeberg, Germany 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 Lutetium-177 or Gallium-68, respectively.

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 (^{177}Lu -PSMA-617, IND # 131966 and ^{68}Ga -PSMA-HBED-CC, IND# 124495).

8.2. Availability

^{177}Lu -J591 (IND# 11613), ^{177}Lu -PSMA-617 (IND# 131966) 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 ^{177}Lu -J591, ^{177}Lu -PSMA-617 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 immune analysis:

Whole blood samples (2.7 mL in each vial) in two 4.5 mL 2.3% sodium citrate tubes (light blue top tube) will be collected for research analysis at specified time points (Screening/baseline and efficacy visit). These samples are collected for research purposes only and will not be billed to the subject. CTC will be stained for calreticulin analyzed for research purposes only.

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 pre-treatment and efficacy visit). 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 and to identify DNA repair and AR-pathway alterations.

9.1.3 Immune Assay:

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. Results of these assays will not be billed to the patient and are for research purposes only.

9.1.4 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 DNA damage repair pathways for research purposes only.

9.2. Special Studies

Conventional Imaging studies as well as ⁶⁸Ga-PSMA-HBED-CC PET/CT imaging and Lutetium-177 Planar/SPECT imaging 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.

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 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 $\geq 30\%$ 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 Duration of CTC response

Duration of CTC response is defined from the first CTC count drop to <5 (or $\leq 50\%$ of baseline) to the time CTC count increases to ≥ 5 (or $\geq 25\%$ increase from nadir).

10.5 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.

10.6 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.

10.7 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.

10.8 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 (⁶⁸Ga-PSMA-HBED-CC PET/CT imaging and Lutetium-177 Planar/SPECT 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 treatment 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 ⁶⁸Ga-PSMA-HBED-CC PET/CT, and those treated with ¹⁷⁷Lu-labeled products will undergo Planar/SPECT imaging. 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 (immune measure)

We will analyze for associations between calreticulin expression as a categorical variable (membrane vs cytoplasmic vs mixed vs no 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 PSMA 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 Phase I Study Design/Endpoints

The phase I portion is a study of subjects with documented progressive metastatic CRPC with the primary endpoint of determination of MTD (or 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 (using 3+3 modified Fibonacci escalation), definitions of DLT, and determination of MTD are defined above (Section 6.2.10). The 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. 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. Phase II Study Design/Endpoints

We will enroll up to 27 subjects into the Phase II portion (including the 3-6 used for establishing the MTD). 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 Simon's two-stage minimax design. We project a 30% PSA decline proportion of 35%, below which the regimen will be unacceptable and a 30% PSA decline proportion of 60%, 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 35% will be tested against the alternative hypothesis that the 30% PSA decline proportion is greater than or equal to 60%.

The sample size computations were performed assuming a 0.10 one-sided level of significance and 90% power assuming a historical control value for PSA decline of 35% and the alternative

hypothesis of 60%. If 6 or fewer of the first 16 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 7 or more patients out of the first 16 evaluable patients experience a 30% decline in PSA, ongoing accrual will proceed to the target sample size of 27 patients (stage 2). The new regimen will be declared active in this patient population and worthy of further testing if 13 or more patients experience a 30% PSA decline among the 27 patients entered. This two-stage design yields a 0.90 probability of a positive result if the true 30% PSA decline proportion is 60%. It yields a 0.90 probability of a negative result if the true 30% PSA decline proportion is 35%. An exact 95% binomial confidence interval will be constructed for the proportion of patients with PSA decline.

12.3 Sample Size/Accrual Rate

The planned sample size for this dose escalation Phase I study is between 6 and 24 total patients and the accrual for phase II will be between 16 and 27 patients and will include patients treated at the MTD from the phase I portion. The accrual rate is anticipated to be 1–2 subjects every month on average (with expected pauses in enrollment between dose-escalation cohorts and between the stage 1 and stage 2 of the phase II trial).

12.4 Stratification Factors

This is not a randomized trial and so there are no stratification factors.

12.5 Analysis of Endpoints

12.5.1 Analysis of Primary Endpoints

The primary endpoint will be the proportion of subjects with DLT from Treatment Visit #1 through End of Study (Efficacy/scan) Visit. The MTD is the highest dose amongst the different dose-level cohorts in this study at which no more than 1 out of 6 subjects in a cohort experience DLT.

12.5.2 Analysis of Secondary Endpoints

For PSA response, CTC response and imaging response, descriptive analysis will be done. For imaging response RECIST (Version 1.1) criteria with PCWG3 modifications will be applied. Biomarker and patient reported outcome data will be analyzed within this study as well as across additional radiolabeled-PSMA studies.

12.6 Interim Analysis

No interim analysis is planned for the phase I portion (though safety analyses will occur in real-time and prior to each dose-escalation). For the phase II portion, accrual will be suspended

after 16 patients have been enrolled in stage 1. If at least 7 patients out of the 16 patients experienced a PSA decline > 30%, 9 more patients will be accrued as part of stage 2.

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 ⁶⁸Ga-PSMA-HBED-CC as well as when they receive their first treatment with ¹⁷⁷Lu-J591 + ¹⁷⁷Lu-PSMA-617 and up to 30 days after their last treatment. 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 ¹⁷⁷Lu-J591 + ¹⁷⁷Lu-PSMA-617 treatment combination (2 doses of each investigational agent) 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 ^{177}Lu -J591, ^{177}Lu -PSMA-617 or ^{68}Ga -PSMA-HBED-CC. Because of the potential for infusion or allergic reaction, the subject should be monitored for safety for one to two hours following the infusion.

Based on prior studies, the known side effects, risks, and hazards associated with the administration of ^{177}Lu -J591, ^{177}Lu -PSMA-617 or ^{68}Ga -PSMA-HBED-CC include: infusion reaction (fever, chills, rash, hypotension, and/or hypertension after injection), xerostomia, renal insufficiency, hematological/bone marrow toxicity (thrombocytopenia, neutropenia) and transient hepatic enzyme elevations. In addition, allergic reactions including anaphylaxis, hypotension, hypertension, difficulty breathing, rash, human anti-humanized antibody (HAHA) response and CNS toxicity are a rare possibility.

Precautions/monitoring:

The infusion of ^{177}Lu -J591, ^{177}Lu -PSMA-617 or ^{68}Ga -PSMA-HBED-CC 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. Vital signs will be monitored before/after the ^{177}Lu -J591 + ^{177}Lu -PSMA-617 or ^{68}Ga -PSMA-HBED-CC 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 infusion, and within 30 minutes and 60 minutes (+/- 10) after the infusion (also, 90 +/- 10 minutes and 120 +/- 10 minutes for ^{177}Lu -J591 + ^{177}Lu -PSMA-617). If any subject has any adverse reaction at 60 minutes for ^{68}Ga -PSMA-HBED-CC and 120 minutes for ^{177}Lu -J591 + ^{177}Lu -PSMA-617, 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:

http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf

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:

http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf

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 and prior to transition from phase I to phase II. 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.

Sarah Rutherford, MD will serve as the Medical Monitor for this study.

15. REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66(1):7-30.
2. Cookson MS, Roth BJ, Dahm P, Engstrom C, Freedland SJ, Hussain M, et al. Castration-resistant prostate cancer: AUA Guideline. *The Journal of urology*. 2013;190(2):429-38.
3. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalthorn TF, Higano CS, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. *Cancer research*. 2008;68(11):4447-54.
4. Mohler JL, Titus MA, Bai S, Kennerley BJ, Lih FB, Tomer KB, et al. Activation of the androgen receptor by intratumoral bioconversion of androstenediol to dihydrotestosterone in prostate cancer. *Cancer research*. 2011;71(4):1486-96.
5. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012;367(13):1187-97.
6. Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, Sternberg CN, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. *The Lancet Oncology*. 2015;16(2):152-60.
7. Fizazi K, Scher HI, Molina A, Logothetis CJ, Chi KN, Jones RJ, et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. *The Lancet Oncology*. 2012;13(10):983-92.
8. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. *N Engl J Med*. 2014;371(5):424-33.
9. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet (London, England)*. 2010;376(9747):1147-54.
10. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):411-22.
11. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus

- prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med*. 2004;351(15):1502-12.
12. Petrylak DP, Tangen CM, Hussain MH, Lara PN, Jr., Jones JA, Taplin ME, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med*. 2004;351(15):1513-20.
 13. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fossa SD, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med*. 2013;369(3):213-23.
 14. Antonarakis ES, Lu C, Wang H, Lubner B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med*. 2014;371(11):1028-38.
 15. Smith MR, Saad F, Coleman R, Shore N, Fizazi K, Tombal B, et al. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: results of a phase 3, randomised, placebo-controlled trial. *Lancet (London, England)*. 2012;379(9810):39-46.
 16. Horoszewicz JS, Kawinski E, Murphy GP. Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. *Anticancer Res*. 1987;7(5B):927-35.
 17. Israeli RS, Powell CT, Fair WR, Heston WD. Molecular cloning of a complementary DNA encoding a prostate-specific membrane antigen. *Cancer Res*. 1993;53(2):227-30.
 18. Israeli RS, Powell CT, Corr JG, Fair WR, Heston WD. Expression of the prostate-specific membrane antigen. *Cancer Res*. 1994;54(7):1807-11.
 19. Wright GL, Jr., Haley C, Beckett ML, Schellhammer PF. Expression of prostate-specific membrane antigen in normal, benign, and malignant prostate tissues. *Urol Oncol*. 1995;1(1):18-28.
 20. Troyer JK, Beckett ML, Wright GL, Jr. Detection and characterization of the prostate-specific membrane antigen (PSMA) in tissue extracts and body fluids. *Int J Cancer*. 1995;62(5):552-8.
 21. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. *Lancet (London, England)*. 2003;361(9361):955-64.
 22. Huang E, Teh BS, Mody DR, Carpenter LS, Butler EB. Prostate adenocarcinoma presenting with inguinal lymphadenopathy. *Urology*. 2003;61(2):463.
 23. Wu LM, Xu JR, Ye YQ, Lu Q, Hu JN. The clinical value of diffusion-weighted imaging in combination with T2-weighted imaging in diagnosing prostate carcinoma: a systematic review and meta-analysis. *AJR American journal of roentgenology*. 2012;199(1):103-10.
 24. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res*. 1997;3(1):81-5.
 25. Bostwick DG, Pacelli A, Blute M, Roche P, Murphy GP. Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: a study of 184 cases. *Cancer*. 1998;82(11):2256-61.
 26. Evans MJ, Smith-Jones PM, Wongvipat J, Navarro V, Kim S, Bander NH, et al. Noninvasive measurement of androgen receptor signaling with a positron-emitting radiopharmaceutical that targets prostate-specific membrane antigen. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(23):9578-82.
 27. Hamilton A, King S, Liu H, Moy P, Bander N, Carr F. A novel humanized antibody

against prostate specific membrane antigen (PSMA) for in vivo targeting and therapy. *Proc Am Assoc Cancer Res* 1998;39:440.

28. Bander NH, Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ. Phase I trial of ¹⁷⁷lutetium-labeled J591, a monoclonal antibody to prostate-specific membrane antigen, in patients with androgen-independent prostate cancer. *J Clin Oncol*. 2005;23(21):4591-601.
29. Galsky MD, Eisenberger M, Moore-Cooper S, Kelly WK, Slovin SF, DeLaCruz A, et al. Phase I trial of the prostate-specific membrane antigen-directed immunoconjugate MLN2704 in patients with progressive metastatic castration-resistant prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2008;26(13):2147-54.
30. Milowsky MI, Nanus DM, Kostakoglu L, Sheehan CE, Vallabhajosula S, Goldsmith SJ, et al. Vascular targeted therapy with anti-prostate-specific membrane antigen monoclonal antibody J591 in advanced solid tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2007;25(5):540-7.
31. Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ, Bander NH. Phase I trial of yttrium-90-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer. *J Clin Oncol*. 2004;22(13):2522-31.
32. Morris MJ, Divgi CR, Pandit-Taskar N, Batraki M, Warren N, Nacca A, et al. Pilot trial of unlabeled and indium-111-labeled anti-prostate-specific membrane antigen antibody J591 for castrate metastatic prostate cancer. *Clin Cancer Res*. 2005;11(20):7454-61.
33. Morris MJ, Pandit-Taskar N, Divgi CR, Bender S, O'Donoghue JA, Nacca A, et al. Phase I evaluation of J591 as a vascular targeting agent in progressive solid tumors. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2007;13(9):2707-13.
34. Vallabhajosula S, Goldsmith SJ, Hamacher KA, Kostakoglu L, Konishi S, Milowski MI, et al. Prediction of myelotoxicity based on bone marrow radiation-absorbed dose: radioimmunotherapy studies using ⁹⁰Y- and ¹⁷⁷Lu-labeled J591 antibodies specific for prostate-specific membrane antigen. *J Nucl Med*. 2005;46(5):850-8.
35. Vallabhajosula S, Goldsmith SJ, Kostakoglu L, Milowsky MI, Nanus DM, Bander NH. Radioimmunotherapy of prostate cancer using ⁹⁰Y- and ¹⁷⁷Lu-labeled J591 monoclonal antibodies: effect of multiple treatments on myelotoxicity. *Clin Cancer Res*. 2005;11(19 Pt 2):7195s-200s.
36. Vallabhajosula S, Kuji I, Hamacher KA, Konishi S, Kostakoglu L, Kothari PA, et al. Pharmacokinetics and biodistribution of ¹¹¹In- and ¹⁷⁷Lu-labeled J591 antibody specific for prostate-specific membrane antigen: prediction of ⁹⁰Y-J591 radiation dosimetry based on ¹¹¹In or ¹⁷⁷Lu? *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2005;46(4):634-41.
37. al. BNe. Phase I clinical trial targeting a monoclonal antibody (mAb) to the extracellular domain of prostate specific membrane antigen (PSMAext) in patients with hormone-independent prostate cancer. *American Society of Clinical Oncology* 2000.
38. Yao V, Parwani A, Maier C, Heston WD, Bacich DJ. Moderate expression of prostate-specific membrane antigen, a tissue differentiation antigen and folate hydrolase, facilitates prostate carcinogenesis. *Cancer research*. 2008;68(21):9070-7.
39. Tagawa ST, Milowsky MI, Morris M, Vallabhajosula S, Christos P, Akhtar NH, et al.

Phase II study of Lutetium-177-labeled anti-prostate-specific membrane antigen monoclonal antibody J591 for metastatic castration-resistant prostate cancer. *Clin Cancer Res.* 2013;19(18):5182-91.

40. Tagawa ST, Vallabhajosula S, Osborne J, Goldsmith SJ, Petrillo K, Tyrell L, et al. Phase I trial of fractionated-dose (177)lutetium radiolabeled anti-specific membrane antigen (PSMA) monoclonal antibody J591 (Lu-177-J591) in patients (pts) with metastatic castration-resistant prostate cancer (metCRPC). *Journal of Clinical Oncology.* 2010;28(15).
41. Scott T, Tagawa JB, Shankar Vallabhajosula, Yuliya Jhanwar, Paul J. Christos, Lauren Emmerich, Beerinder S. Karir, Irene Karpenko, Linda Lam, Victor RA Febles, Ana M. Molina, Himisha Beltran, Stanley J. Goldsmith, Neil Harrison Bander, David M. Nanus. Final results of 2-dose fractionation of 177Lu-J591 for progressive metastatic castration-resistant prostate cancer (mCRPC). *J Clin Oncol.* 2016;34:(suppl; abstr 5022).
42. Batra JS, Karir BS, Vallabhajosula S, Christos PJ, Hodes G, Date PR, et al., editors. Fractionated dose radiolabeled antiprostate specific membrane antigen (PSMA) radioimmunotherapy (177Lu-J591) with or without docetaxel for metastatic castration-resistant prostate cancer (mCRPC). *ASCO Annual Meeting Proceedings;* 2015.
43. Deb N, Goris M, Trisler K, Fowler S, Saal J, Ning S, et al. Treatment of hormone-refractory prostate cancer with 90Y-CYT-356 monoclonal antibody. *Clin Cancer Res.* 1996;2(8):1289-97.
44. Yadav MP, Ballal S, Tripathi M, Damle NA, Sahoo RK, Seth A, et al. 177Lu-DKFZ-PSMA-617 therapy in metastatic castration resistant prostate cancer: safety, efficacy, and quality of life assessment. *Eur J Nucl Med Mol Imaging.* 2016.
45. Rahbar K, Bode A, Weckesser M, Avramovic N, Claesener M, Stegger L, et al. Radioligand Therapy With 177Lu-PSMA-617 as A Novel Therapeutic Option in Patients With Metastatic Castration Resistant Prostate Cancer. *Clin Nucl Med.* 2016;41(7):522-8.
46. Kratochwil C, Giesel FL, Stefanova M, Benesova M, Bronzel M, Afshar-Oromieh A, et al. PSMA-Targeted Radionuclide Therapy of Metastatic Castration-Resistant Prostate Cancer with 177Lu-Labeled PSMA-617. *J Nucl Med.* 2016;57(8):1170-6.
47. Kabasakal L, AbuQbeith M, Aygun A, Yeyin N, Ocak M, Demirci E, et al. Pre-therapeutic dosimetry of normal organs and tissues of (177)Lu-PSMA-617 prostate-specific membrane antigen (PSMA) inhibitor in patients with castration-resistant prostate cancer. *Eur J Nucl Med Mol Imaging.* 2015;42(13):1976-83.
48. Baum RP, Kulkarni HR, Schuchardt C, Singh A, Wirtz M, Wiessalla S, et al. 177Lu-Labeled Prostate-Specific Membrane Antigen Radioligand Therapy of Metastatic Castration-Resistant Prostate Cancer: Safety and Efficacy. *J Nucl Med.* 2016;57(7):1006-13.
49. Rahbar K, Ahmadzadehfar H, Kratochwil C, Haberkorn U, Schäfers M, Essler M, et al. German multicenter study investigating 177Lu-PSMA-617 radioligand therapy in advanced prostate cancer patients. *Journal of Nuclear Medicine.* 2017;58(1):85-90.
50. Afshar-Oromieh A, Zechmann CM, Malcher A, Eder M, Eisenhut M, Linhart HG, et al. Comparison of PET imaging with a (68)Ga-labelled PSMA ligand and (18)F-choline-based PET/CT for the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging.* 2014;41(1):11-20.
51. Afshar-Oromieh A, Haberkorn U, Hadaschik B, Habl G, Eder M, Eisenhut M, et al. PET/MRI with a 68Ga-PSMA ligand for the detection of prostate cancer. *Eur J Nucl Med Mol*

Imaging. 2013;40(10):1629-30.

52. Afshar-Oromieh A, Haberkorn U, Eder M, Eisenhut M, Zechmann CM. [68Ga]Gallium-labelled PSMA ligand as superior PET tracer for the diagnosis of prostate cancer: comparison with 18F-FECH. *Eur J Nucl Med Mol Imaging*. 2012;39(6):1085-6.
53. Eiber M, Nekolla SG, Maurer T, Weirich G, Wester HJ, Schwaiger M. (68)Ga-PSMA PET/MR with multimodality image analysis for primary prostate cancer. *Abdominal imaging*. 2015;40(6):1769-71.
54. Uprimny C, Kroiss A, Nilica B, Buxbaum S, Decristoforo C, Horninger W, et al. (68)Ga-PSMA ligand PET versus (18)F-NaF PET: evaluation of response to (223)Ra therapy in a prostate cancer patient. *Eur J Nucl Med Mol Imaging*. 2015;42(2):362-3.
55. Chakraborty PS, Tripathi M, Agarwal KK, Kumar R, Vijay MK, Bal C. Metastatic poorly differentiated prostatic carcinoma with neuroendocrine differentiation: negative on 68Ga-PSMA PET/CT. *Clin Nucl Med*. 2015;40(2):e163-6.
56. Barrett JA, Coleman RE, Goldsmith SJ, Vallabhajosula S, Petry NA, Cho S, et al. First-in-man evaluation of 2 high-affinity PSMA-avid small molecules for imaging prostate cancer. *J Nucl Med*. 2013;54(3):380-7.
57. Hillier SM, Maresca KP, Femia FJ, Marquis JC, Foss CA, Nguyen N, et al. Preclinical evaluation of novel glutamate-urea-lysine analogues that target prostate-specific membrane antigen as molecular imaging pharmaceuticals for prostate cancer. *Cancer Res*. 2009;69(17):6932-40.
58. Eder M, Neels O, Muller M, Bauder-Wust U, Remde Y, Schafer M, et al. Novel Preclinical and Radiopharmaceutical Aspects of [68Ga]Ga-PSMA-HBED-CC: A New PET Tracer for Imaging of Prostate Cancer. *Pharmaceuticals (Basel, Switzerland)*. 2014;7(7):779-96.
59. Eder M, Schafer M, Bauder-Wust U, Hull WE, Wangler C, Mier W, et al. 68Ga-complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging. *Bioconjugate chemistry*. 2012;23(4):688-97.
60. Afshar-Oromieh A, Haberkorn U, Schlemmer HP, Fenchel M, Eder M, Eisenhut M, et al. Comparison of PET/CT and PET/MRI hybrid systems using a 68Ga-labelled PSMA ligand for the diagnosis of recurrent prostate cancer: initial experience. *Eur J Nucl Med Mol Imaging*. 2014;41(5):887-97.
61. Afshar-Oromieh A, Avtzi E, Giesel FL, Holland-Letz T, Linhart HG, Eder M, et al. The diagnostic value of PET/CT imaging with the (68)Ga-labelled PSMA ligand HBED-CC in the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging*. 2015;42(2):197-209.
62. Eiber M, Maurer T, Souvatzoglou M, Beer AJ, Ruffani A, Haller B, et al. Evaluation of Hybrid (6)(8)Ga-PSMA Ligand PET/CT in 248 Patients with Biochemical Recurrence After Radical Prostatectomy. *J Nucl Med*. 2015;56(5):668-74.
63. Perera M, Papa N, Christidis D, Wetherell D, Hofman MS, Murphy DG, et al. Sensitivity, Specificity, and Predictors of Positive 68Ga-Prostate-specific Membrane Antigen Positron Emission Tomography in Advanced Prostate Cancer: A Systematic Review and Meta-analysis. *Eur Urol*. 2016.
64. Afshar-Oromieh A, Malcher A, Eder M, Eisenhut M, Linhart HG, Hadaschik BA, et al. PET imaging with a [68Ga]gallium-labelled PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumour lesions. *Eur J Nucl Med Mol Imaging*. 2013;40(4):486-95.

65. Hamilton AA, Liu H, Moy P, Bander N, Carr F. A novel humanized antibody against prostate specific membrane antigen (PSMA) for in vivo targeting and therapy. *Proc Am Assoc for Cancer Res.* 1998;39:440.
66. Langmuir VK, Fowler JF, Knox SJ, Wessels BW, Sutherland RM, Wong JY. Radiobiology of radiolabeled antibody therapy as applied to tumor dosimetry. *Medical physics.* 1993;20(2 Pt 2):601-10.
67. Leichner PK, Akabani G, Colcher D, Harrison KA, Hawkins WG, Eckblade M, et al. Patient-specific dosimetry of indium-111- and yttrium-90-labeled monoclonal antibody CC49. *J Nucl Med.* 1997;38(4):512-6.
68. DeNardo SJ, Kramer EL, O'Donnell RT, Richman CM, Salako QA, Shen S, et al. Radioimmunotherapy for breast cancer using indium-111/yttrium-90 BrE-3: results of a phase I clinical trial. *J Nucl Med.* 1997;38(8):1180-5.
69. Benesova M, Schafer M, Bauder-Wust U, Afshar-Oromieh A, Kratochwil C, Mier W, et al. Preclinical Evaluation of a Tailor-Made DOTA-Conjugated PSMA Inhibitor with Optimized Linker Moiety for Imaging and Endoradiotherapy of Prostate Cancer. *J Nucl Med.* 2015;56(6):914-20.
70. Kratochwil C, Giesel FL, Eder M, Afshar-Oromieh A, Benesova M, Mier W, et al. [(1)(7)(7)Lu]Lutetium-labelled PSMA ligand-induced remission in a patient with metastatic prostate cancer. *Eur J Nucl Med Mol Imaging.* 2015;42(6):987-8.
71. Ahmadzadehfar H, Rahbar K, Kurpig S, Bogemann M, Claesener M, Eppard E, et al. Early side effects and first results of radioligand therapy with (177)Lu-DKFZ-617 PSMA of castrate-resistant metastatic prostate cancer: a two-centre study. *EJNMMI Res.* 2015;5(1):114.
72. Bill-Axelsson A, Holmberg L, Filen F, Ruutu M, Garmo H, Busch C, et al. Radical prostatectomy versus watchful waiting in localized prostate cancer: the Scandinavian prostate cancer group-4 randomized trial. *Journal of the National Cancer Institute.* 2008;100(16):1144-54.
73. Isbarn H, Wanner M, Salomon G, Steuber T, Schlomm T, Kollermann J, et al. Long-term data on the survival of patients with prostate cancer treated with radical prostatectomy in the prostate-specific antigen era. *BJU international.* 2010;106(1):37-43.
74. Bolla M, van Poppel H, Tombal B, Vekemans K, Da Pozzo L, de Reijke TM, et al. Postoperative radiotherapy after radical prostatectomy for high-risk prostate cancer: long-term results of a randomised controlled trial (EORTC trial 22911). *Lancet (London, England).* 2012;380(9858):2018-27.
75. Thompson IM, Jr., Tangen CM, Paradelo J, Lucia MS, Miller G, Troyer D, et al. Adjuvant radiotherapy for pathologically advanced prostate cancer: a randomized clinical trial. *Jama.* 2006;296(19):2329-35.
76. Messing EM, Manola J, Yao J, Kiernan M, Crawford D, Wilding G, et al. Immediate versus deferred androgen deprivation treatment in patients with node-positive prostate cancer after radical prostatectomy and pelvic lymphadenectomy. *The Lancet Oncology.* 2006;7(6):472-9.
77. Shelley MD, Kumar S, Wilt T, Staffurth J, Coles B, Mason MD. A systematic review and meta-analysis of randomised trials of neo-adjuvant hormone therapy for localised and locally advanced prostate carcinoma. *Cancer treatment reviews.* 2009;35(1):9-17.
78. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5):646-74.

79. Coffey DS, Isaacs JT. Prostate tumor biology and cell kinetics--theory. *Urology*. 1981;17(Suppl 3):40-53.
80. Sweat SD, Pacelli A, Murphy GP, Bostwick DG. Prostate-specific membrane antigen expression is greatest in prostate adenocarcinoma and lymph node metastases. *Urology*. 1998;52(4):637-40.
81. Wright GL, Jr., Grob BM, Haley C, Grossman K, Newhall K, Petrylak D, et al. Upregulation of prostate-specific membrane antigen after androgen-deprivation therapy. *Urology*. 1996;48(2):326-34.
82. Griffin MM, Morley N. Rituximab in the treatment of non-Hodgkin's lymphoma--a critical evaluation of randomized controlled trials. *Expert opinion on biological therapy*. 2013;13(5):803-11.
83. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE, Jr., Davidson NE, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *The New England journal of medicine*. 2005;353(16):1673-84.
84. Tomlins SA, Mehra R, Rhodes DR, Cao X, Wang L, Dhanasekaran SM, et al. Integrative molecular concept modeling of prostate cancer progression. *Nature genetics*. 2007;39(1):41-51.
85. Lapointe J, Li C, Higgins JP, van de Rijn M, Bair E, Montgomery K, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101(3):811-6.
86. Hendriksen PJ, Dits NF, Kokame K, Veldhoven A, van Weerden WM, Bangma CH, et al. Evolution of the androgen receptor pathway during progression of prostate cancer. *Cancer research*. 2006;66(10):5012-20.
87. Miyamoto DT, Lee RJ, Stott SL, Ting DT, Wittner BS, Ulman M, et al. Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer. *Cancer discovery*. 2012;2(11):995-1003.
88. al. STTe. Noninvasive measurement of prostate-specific membrane antigen (PSMA) expression with radiolabeled J591 imaging: A prognostic tool for metastatic castration-resistant prostate cancer (CRPC). *Journal of clinical Oncology* 2013.
89. Bander NH, Trabulsi EJ, Kostakoglu L, Yao D, Vallabhajosula S, Smith-Jones P, et al. Targeting metastatic prostate cancer with radiolabeled monoclonal antibody J591 to the extracellular domain of prostate specific membrane antigen. *J Urol*. 2003;170(5):1717-21.
90. Nanus DM, Milowsky MI, Kostakoglu L, Smith-Jones PM, Vallabahajosula S, Goldsmith SJ, et al. Clinical use of monoclonal antibody HuJ591 therapy: targeting prostate specific membrane antigen. *J Urol*. 2003;170(6 Pt 2):S84-8; discussion S8-9.
91. Tagawa S, Milowsky M, Morris M, Vallabhajosula S, Goldsmith S, Matulich D, et al. Phase II trial of 177Lutetium radiolabeled anti-prostate-specific membrane antigen (PSMA) monoclonal antibody J591 (177Lu-J591) in patients (pts) with metastatic castrate-resistant prostate cancer (metCRPC). *Journal of Clinical Oncology*. 2008;26(90150):5140-.
92. Tagawa S, Vallabhajosula S, Osborne J, Goldsmith S, Petrillo K, Tyrell L, et al. Phase I trial of fractionated-dose 177lutetium radiolabeled anti-prostate-specific membrane antigen (PSMA) monoclonal antibody J591 (177Lu-J591) in patients (pts) with metastatic castration-resistant prostate cancer (metCRPC). *Journal of Clinical Oncology*. 2010;28(15_suppl):4667-.
93. Tagawa ST, Whang YE, Kaur G, Vallabhajosula S, Christos PJ, Nikolopoulou A, et al. Phase I trial of docetaxel/prednisone plus fractionated dose radiolabeled anti-prostate-specific

- membrane antigen (PSMA) monoclonal antibody 177lu-J591 in patients with metastatic, castration-resistant prostate cancer (mCRPC). American Society of Clinical Oncology; 2014.
94. Meyn RE. Apoptosis and response to radiation: implications for radiation therapy. *Oncology* (Williston Park, NY). 1997;11(3):349-56; discussion 56, 61, 65.
 95. Mirzaie-Joniani H, Eriksson D, Johansson A, Lofroth PO, Johansson L, Ahlstrom KR, et al. Apoptosis in HeLa Hep2 cells is induced by low-dose, low-dose-rate radiation. *Radiation research*. 2002;158(5):634-40.
 96. Kroger LA, DeNardo GL, Gumerlock PH, Xiong CY, Winthrop MD, Shi XB, et al. Apoptosis-related gene and protein expression in human lymphoma xenografts (Raji) after low dose rate radiation using ⁶⁷Cu-2IT-BAT-Lym-1 radioimmunotherapy. *Cancer biotherapy & radiopharmaceuticals*. 2001;16(3):213-25.
 97. DeNardo GL, Schlom J, Buchsbaum DJ, Meredith RF, O'Donoghue JA, Sgouros G, et al. Rationales, evidence, and design considerations for fractionated radioimmunotherapy. *Cancer*. 2002;94(4 Suppl):1332-48.
 98. Burke PA, DeNardo SJ, Miers LA, Kukis DL, DeNardo GL. Combined modality radioimmunotherapy. Promise and peril. *Cancer*. 2002;94(4 Suppl):1320-31.
 99. Larson SM, Carrasquillo JA, Cheung NK, Press OW. Radioimmunotherapy of human tumours. *Nature reviews Cancer*. 2015;15(6):347-60.
 100. Ali AM, Dehdashti F, DiPersio JF, Cashen AF. Radioimmunotherapy-based conditioning for hematopoietic stem cell transplantation: Another step forward. *Blood reviews*. 2016;30(5):389-99.
 101. Shen S, Duan J, Meredith RF, Buchsbaum DJ, Brezovich IA, Pareek PN, et al. Model prediction of treatment planning for dose-fractionated radioimmunotherapy. *Cancer*. 2002;94(4 Suppl):1264-9.
 102. O'Donoghue JA, Sgouros G, Divgi CR, Humm JL. Single-dose versus fractionated radioimmunotherapy: model comparisons for uniform tumor dosimetry. *J Nucl Med*. 2000;41(3):538-47.
 103. Vriesendorp HM, Shao Y, Blum JE, Quadri SM, Williams JR. Fractionated intravenous administration of ⁹⁰Y-labeled B72.3 GYK-DTPA immunoconjugate in beagle dogs. *Nuclear medicine and biology*. 1993;20(5):571-8.
 104. Buchsbaum D, Khazaeli MB, Liu T, Bright S, Richardson K, Jones M, et al. Fractionated radioimmunotherapy of human colon carcinoma xenografts with ¹³¹I-labeled monoclonal antibody CC49. *Cancer Res*. 1995;55(23 Suppl):5881s-7s.
 105. DeNardo GL, DeNardo SJ, Lamborn KR, Goldstein DS, Levy NB, Lewis JP, et al. Low-dose, fractionated radioimmunotherapy for B-cell malignancies using ¹³¹I-Lym-1 antibody. *Cancer biotherapy & radiopharmaceuticals*. 1998;13(4):239-54.
 106. Steffens MG, Boerman OC, Oyen WJ, Kniest PH, Witjes JA, Oosterhof GO, et al. Intratumoral distribution of two consecutive injections of chimeric antibody G250 in primary renal cell carcinoma: implications for fractionated dose radioimmunotherapy. *Cancer Res*. 1999;59(7):1615-9.
 107. Hindorf C, Linden O, Stenberg L, Tennvall J, Strand SE. Change in tumor-absorbed dose due to decrease in mass during fractionated radioimmunotherapy in lymphoma patients. *Clin Cancer Res*. 2003;9(10 Pt 2):4003s-6s.
 108. Rahbar K, Schmidt M, Heinzl A, Eppard E, Bode A, Yordanova A, et al. Response and

tolerability of a single dose of 177Lu-PSMA-617 in patients with metastatic castration-resistant prostate cancer: a multicenter retrospective analysis. J Nucl Med. 2016.

109. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(19):6302-9.

110. Scher HI, Heller G, Molina A, Attard G, Danila DC, Jia X, et al. Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. J Clin Oncol. 2015;33(12):1348-55.

111. Miyamoto DT, Lee RJ, Stott SL, Ting DT, Wittner BS, Ulman M, et al. Androgen Receptor Signaling in Circulating Tumor Cells as a Marker of Hormonally Responsive Prostate Cancer. Cancer Discovery. 2012;2(11):995-1003.

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 | 20 | Very sick, hospitalization indicated. Death not imminent. |

| | | | |
|----------|----------------------------------|-----------|---|
| | confined to bed or chair. | 10 | Moribund, fatal processes progressing rapidly. |
| 5 | Dead. | 0 | Dead. |

APPENDIX B

WCMC IRB SAE Reporting Forms

http://researchintegrity.weill.cornell.edu/institutional_review_board/irb_adv.html