TITLE: PSMA Imaging of Localized Prostate Cancer

Principal Investigator: *Douglas Scherr, MD*

[redacted]

Co-Investigators:

[redacted]

Statistician:

[redacted]

Study Nurse:

[redacted]

Study Coordinator:

[redacted]

Study Coordinator:

[redacted]

Protocol Version Date:
Original, Version 1, dated May 6, 2015
Amendment1, Version 2, dated July 16, 2015
Amendment 2, Version 3, dated May 14, 2016
Amendment 3, Version 4, dated December 22, 2016
Amendment 4, Version 5 dated January 27, 2017
Amendment 5, Version 6 dated May 31, 2017

SCHEMA

ENROLLMENT

Twenty (20) subjects in total will be enrolled at Weill Cornell Medical College (WCMC)/ NYPH.

IMAGING

After the screening period (up to 28 days), each subject will be scheduled to receive 10 mg injection of df-IAB2M conjugated with 2.5 mCi of ^{89}Zr.

Subjects will undergo ^{89}Zr-df-IAB2M PET/CT scan 2-4 days after the injection of ^{89}Zr-df-IAB2M depending on patient's availability and compliance. Images read by a Nuclear Medicine Physician reporting: location, SUV and, if possible, size of all areas with abnormal uptake.

Optional but recommended ^{68}Ga-PSMA-HBED-CC (5±2mCi) injection and PET/CT scan (1 to 3 hours after the injection) will also be performed prior to radical prostatectomy depending on subject's availability and compliance.

(subjects will also undergo a pelvic MRI if they have not obtained an MR image during the screening period or on day of infusion)

RADICAL PROSTATECTOMY

3-30 days after ^{89}Zr-df-IAB2M PET/CT scan (preferably 1 week after the scan), depending on patient's availability and compliance.

HISTOPATHOLOGY

Including individual lesion mapping, size determination, Gleason score and IHC findings.

RESULTS

Histopathology lesion data correlated with imaging results.

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1. OBJECTIVES

1.1. Primary Objectives

- To evaluate the ability of ^{89}Zr-df-IAB2M to detect localized, clinically significant (defined as: ≥ 0.5 cm^3 with Gleason pattern ≥ 4) prostate cancer (PCa).

1.2. Secondary Objectives

- To determine the correlation of ^{89}Zr-df-IAB2M PET/CT findings with pathological features (i.e. Gleason pattern, diameter and PSMA-positivity).
- To compare the *in vivo* ^{89}Zr-df-IAB2M PET/CT and ^{68}Ga-PSMA-HBED-CC PET/CT to mpMRI.
- To determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of IAB2M to detect all pathologically identified PCa lesions that do not meet criteria for clinical significance (defined as: < 0.5 cm^3 with Gleason pattern < 4).
- Determine the proportion of IAB2M positivity (as measured by SUV) versus lesion size, Gleason pattern, and PSA levels.
- Determine ability of ^{89}Zr-df-IAB2M PET/CT to identify extra-prostatic extension.
- Determine ability of ^{89}Zr-df-IAB2M PET/CT to identify lymph node or other extra-prostatic involvement.
- To determine the safety of ^{89}Zr-df-IAB2M PET/CT and 68Ga-PSMA-HBED-CC PET/CT imaging.

2. BACKGROUND

2.1 Disease

Localized PCa is presently evaluated by serum prostate specific antigen (PSA) sampling and transrectal ultrasound (TRUS) and magnetic resonance (MR) imaging, both of the latter being anatomic approaches. As a secretory protein, PSA does not provide anatomic details nor does it distinguish between malignant or benign pathology. TRUS and MR imaging modalities are limited by their inability to identify and differentiate localized malignant tissue from common benign pathology, i.e., benign prostatic hyperplasia, inflammation, etc. Furthermore, the images created are poorly defined and subject to artifact. The present inability to clearly image and identify malignant tissue and to localize the site/s of disease leads to unnecessary biopsy of many patients [1,000,000 prostate biopsies per year in US] and an inability to direct the biopsy towards any suspicious site/s. As a direct result, the substantial majority of prostate biopsies are negative, suggesting that there was no need for the biopsy or that the biopsy failed to sample the lesion[1,2]. Nevertheless, the biopsied patients are subject to potentially significant morbidity and even mortality[3,4].

An imaging modality that is able to selectively identify malignant prostatic tissue within the

gland, would allow for accurate, targeted biopsies of suspicious foci and would reduce unnecessary biopsies in patients with benign pathologies. The modality would also allow for improved clinical staging and risk stratification, improved monitoring of patients on active surveillance and improved targeting of focal therapy.

Prostate Specific Membrane Antigen (PSMA)

PSMA is the single, most well-established, highly restricted prostate epithelial cell membrane antigen known[5-12]. Although first thought to be entirely prostate-specific[5,13,14], subsequent studies demonstrated that PSMA is also expressed by some brain astrocytes and cells of the small intestine, proximal renal tubules and salivary glands[9]. However, the level of expression in these non-prostate tissues is between a hundred-fold to a thousand-fold less than in prostate tissue[8] and the site of PSMA expression in these normal cells (brush border/luminal location) are not readily accessible to circulating antibodies because of the blood: brain barrier or, in the case of the epithelial locations, due to intervening cell layers, basement membrane and epithelial tight junctions.

In contrast to other well-known prostate-restricted molecules such as PSA and prostatic acid phosphatase (PAP), which are secretory proteins, PSMA is a type II integral cell-surface membrane protein that is not secreted. Pathology studies indicate that PSMA is expressed by virtually all prostate cancers[15] and PSMA expression increases progressively in higher grade cancers, metastatic disease, and hormone-refractory prostate cancer[7,9,10]. These qualities make PSMA an ideal target for monoclonal antibody (mAb) imaging and/or therapy.

2.2 Investigational Agent

Humanized J591 (huJ591)

The huJ591 mAb is a de-immunized monoclonal antibody directed at the extracellular domain of human PSMA. huJ591 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.[16] 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. huJ591 is produced from NS0 cells by Lonza (Lonza Biologics, Slough, UK).

The humanized version of J591 has provided promising results in imaging both localized PCa and metastatic disease. Initial phase I/II studies using huJ591 trace-labeled with ^{111}Indium (^{111}In) and ^{177}Lutitium (^{177}Lu) using a DOTA chelate showed that repetitive dosing was well tolerated without the development of a human anti-humanized antibody (HAHA) response[17-22]. 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 PCa 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 huJ591, and higher doses were associated with longer plasma clearance times.[23-25]

Humanized J591-Minibody (IAB2M)

IAB2M is an engineered antibody fragment (derived from huJ591) that targets the extracellular domain of the PSMA. The intact parental huJ591 antibody demonstrated sufficient tumor uptake to be useful as an imaging and therapy agent[17,26-29], however, the prolonged blood circulation time leads to increased background activity and radiation dose to normal organs thereby, somewhat limiting the imaging or therapeutic potential of these agents. Radiolabeled engineered antibody fragments have much faster clearance rates, providing improved tumor-to-blood ratios[30,31]. However, fragments below 60 kDa are filtered through the glomerulus, leading to significant renal excretory activity[32], which is an unfavorable characteristic for a PCa imaging agent as the radioactivity within the bladder interferes with imaging of the adjacent prostate. To address these issues, IAB2M was engineered by fusing VH-linker-VL-CH3 gene segments, producing a single-chain protein that self-assembles into a small, stable, bivalent imaging agent with a molecular weight of approximately 80 kDa. The minibody format is half the size of complete, full-length antibodies, yet retains full bivalent binding to the target and is not excreted via the glomerulus.

Previous studies with a radiolabeled minibody demonstrates rapid biodistribution, efficient target penetration, and rapid blood/background clearance due to the smaller size, allowing high-contrast images to be obtained within hours after injection[33-35] compared to the current delay of several days to a week when intact antibodies are used for imaging[36]. The lack of CH2 domain in the minibody format removes antibody effector functions, removes FcRn-mediated prolongation of plasma half-life, and the risk of an immunologic response such as complement-dependent cytotoxicity is reduced to negligible. For functional imaging purposes, IAB2M is conjugated to desferrioxamine (Df) and radiometal labeled with ^{89}Zr (T ½ 78.41 hours). The end product, ^{89}Zr-df-IAB2M, is non-immunogenic, recognizes an extracellular target, is not excreted via the kidney, and exhibits rapid kinetics desirable for imaging.

Engineering the IAB2M Minibody

The DNA sequences of the heavy and light chains for the humanized anti-PSMA antibody huJ591 were provided by Dr. Neil H. Bander (Weill Cornell Medical College). ImaginAb, the creators and IND owners of IAB2M, re-humanized the DNA sequences using a later generation approach. The variable genes from the heavy and light chain DNA were separately amplified and joined together by overlap PCR to form a single-chain Fv (scFv) with an 18 amino acid linker. This scFv fragment was subsequently fused to the human IgG1 CH3 domain via the human IgG1 hinge sequence by overlap PCR to form the complete minibody (scFv-CH3 dimer) construct (see Figure 1).

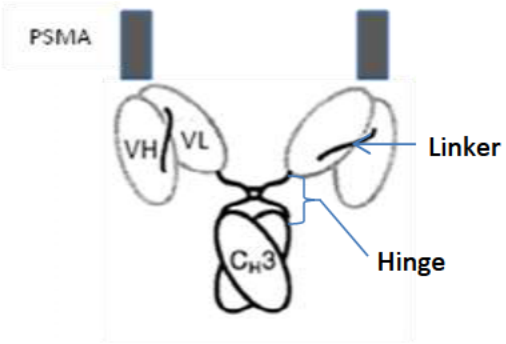


Figure 1. The anti-PSMA minibody is a covalent bound dimer of two single-chains composed of a scFv fused to the human IgG1 hinge-CH3 region. The scFv is the huJ591 variable heavy chain (VH) and light chain (VL) connected by a linker. PSMA, the target antigen, is represented by the gray rectangles at the top.

A cGMP Master Cell Bank (MCB) was created from a stable cell pool at Catalent BioPharma (Middleton, WI). IAB2M was developed in their proprietary GPEx® technology, a high-titer retroviral vector that generated a stable CHO cell line. Full MCB testing has been completed, along with viral clearance. Subsequently a cGMP production run was initiated in the GMP suite from the MCB for the production of IAB2M. IAB2M was released by Catalent BioPharma against qualified release assays for conjugation. cGMP grade Df was obtained (Macrocyclics, Dallas, TX). In the GMP suite at Isotherapeutics Group (Angleton, TX), Df-IAB2M was manufactured and sent out for final safety as well as release testing. In the GMP suite, vials of Df-IAB2M were filled and finished.

Pre-clinical Studies: Functional Characterization of IAB2M

Full characterization of IAB2M was carried out, including a comparison with huJ591. IAB2M binding to PSMA has been confirmed by ELISA and flow cytometry in competition with huJ591. In vitro, *ex-vivo* and *in vivo* internalization assays demonstrate internalization that is comparable to huJ591 (unpublished data).

Serial PET/CT studies on mice with PSMA+ and PSMA- xenografts showed up to 13.25%ID/g uptake in PSMA+, as compared with nominal uptake in PSMA- xenografts. Background activity was observed in the liver (15-20%ID/g), as expected, since the primary route of clearance for minibodies and radiometals is hepatic. Small animal PET/CT studies comparing a labile (I-124) versus residualizing (radiometal/chelater) radiolabeling strategy indicates that as much as 75% of IAB2M is internalized over a 48-hour time period (unpublished data).

Amendment v3 (01/27/17):

^{68}Ga-PSMA-HBED-CC (^{68}Ga-PSMA)

PSMA has recently been discovered as a promising target for radioisotope based approaches, both for PET imaging, for example, using ^{68}Ga-labeled PSMA ligands[37-42]. 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[43,44]. One of these ligands, the small molecule Glu-NH-CO-NH-Lys-(Ahx)-[^{68}Ga(HBED-CC)], also known as PSMA-11®, PSMAHBED, Glu-CO-Lys(Ahx)-HBED-CC, DKFZ-PSMA-11, PSMA-HBED- CC, PSMA-HBED, PSMA or Prostamedix™, developed at the German Cancer Research Center Heidelberg (DKFZ), has become the most clinically used radiotracer. This compound shows a strong binding affinity to PSMA as well as a highly efficient internalization into PCa cells[45,46]. PET/CT-imaging with ^{68}Ga-PSMA-11 has demonstrated this novel method as an important imaging modality for diagnosing recurrent PCa[37,47-49]. Perera et al., did systematic review of ^{68}Ga-PET articles and Sixteen articles involving 1309 patients were analyzed. On per-patient analysis, the summary sensitivity and specificity were both 86%. On per lesion analysis, the summary sensitivity and specificity were 80% and 97%, respectively[50].

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 ^{68}Ga with the final product being ^{68}Ga-PSMA-HBED-CC. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine.

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

2.3 Rationale

^{89}Zr-df-IAB2M for PCa imaging/diagnosis

An ad-hoc analysis of data from 18 subjects with metastatic PCa showed that the combination of conventional imaging (CT, bone scan, FDG PET) and ^{89}Zr-df-IAB2M PET imaging identified a total of 168 lesions (152 bone lesions and 16 soft tissue lesions). Thirty-four of these 168 lesions (32 bone lesions and two soft tissues lesions) were identified by ^{89}Zr-df-IAB2M PET but not by conventional imaging. A total of 13 biopsies in these 18 patients confirmed true-positive identification of lesions in 11 of 11 locations and true-negative identification in one of two locations using ^{89}Zr-df-IAB2M compared with nine of 11 positive lesions and two of two negative lesions correctly identified by FDG PET. This improved sensitivity and specificity illustrates the potential of ^{89}Zr-df-IAB2M PET to provide clinicians with actionable information that may improve the accuracy of diagnosis, staging of the disease, risk stratification, and treatment decision-making.

Further review of ^{89}Zr-df-IAB2M PET/CT by an independent third-party core lab was undertaken to determine the ideal unlabeled protein dose(10, 20 and 50 mg) and timing of

optimal imaging for lesion detection. This un-blinded analysis revealed predominantly pelvic and lumbar spinal bone metastases and nodal metastases detection in the 18 subject cohort. Based on both estimated lesion detection rate, PET/CT activity and reader preference, the 48 hour imaging time point was determined to be the best time point for lesion detection and quantitation, while the time points from 24 hours through 96 hours demonstrated comparable qualitative and quantitative results to the 48 hour time point. Increasing unlabeled antibody affected the time course of detection of prostate metastases in all tissues examined, with the 10 mg cohort demonstrating the most sensitivity for lesion detection and quantitation. These results suggested that ^{89}Zr-df-IAB2M can be an effective probe for the PCa marker PSMA and provide a robust imaging scan to better detect PCa tumor burden.

Amendment v3 (01/27/17):

^{68}Ga-PSMA-HBED-CC for PCa imaging/diagnosis:

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

a) Diagnostic value of ^{68}Ga-PSMA-HBED-CC PET/CT imaging in the diagnosis of recurrent prostate cancer[48]:

The study reports data from a retrospective analysis of 319 patients who underwent ^{68}Ga-PSMA-ligand PET/CT from 2011 to 2014. The ^{68}Ga-PSMA-HBED-CC solution was applied to the respective patient via an intravenous bolus injection (mean of 172.4 MBq ±70.9, range 40 – 400 MBq, median 161 MBq). A non-contrast-enhanced CT scan was performed 1-hour post tracer injection. Histological verification was performed in 42 patients after the ^{68}Ga-PSMA-ligand PET/CT. Tracer uptake was measured in 901 representative tumor lesions. 82.8% of the patients had at least one lesion indicative of PCa was detected. Tumor-detection was positively associated with PSA level and Androgen deprivation therapy (ADT). Gelason Score and PSA doubling time (PSA-DT) were not associated with tumor-detection. The average maximum standardized uptake value (SUVmax) of tumor lesions was 13.3 ± 14.6 (0.7-122.5). Amongst lesions investigated by histology, 30 were false-negative in 4 different patients, and all other lesions (n = 416) were true-positive or true-negative. A lesion-based analysis of sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) revealed values of 76.6%, 100%, 91.4% and 100%. A patient-based analysis revealed a sensitivity of 88.1%.

b) PET imaging with a ^{68}Ga-PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumor lesions [51]:

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.

c) ^{68}Ga-labeled PSMA ligand as superior PET tracer for the diagnosis of prostate cancer: Comparison with ^{18}F-FECH [39]:

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.

d) 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 [47]:

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 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 PCa marker “PSMA” in metastatic prostate cancer patients and that it can provide a robust imaging scan to better detect PCa tumor burden. We plan to utilize this probe to

better understand its diagnostic role in prostate cancer patients with localized disease.

2.4 Correlative Studies Background

Imaging Modalities for the evaluation of Prostate Cancer

Cross-Sectional Imaging

The cross-sectional imaging modalities that are used to evaluate PCa include CT and MRI. The utility of each of these modalities varies according to the stage of disease. At present, neither of these modalities can reliably distinguish viable PCa from necrotic or nonmalignant tissue.

CT: Does not effectively evaluate disease that is confined to the prostate and adjacent area. It is not sensitive enough to detect microscopic nodal disease or tumor in non-enlarged lymph nodes.

MRI: To provide the most accurate assessment of the extra-prostatic region, neurovascular bundles, and seminal vesicles, MRI is considered the modality of choice. However, MRI is limited by multiple sophisticated post-imaging processes and until recently has not been standardized[52]. MRI can detect soft tissue disease and is most useful to corroborate bone scintigraphy findings (particularly when the earliest involvement is thought to be present in the bone marrow). MRI is most sensitive in the axial skeleton and pelvis, and is less effective in the ribs, chest wall, and skull. Multiple studies have demonstrated the inability of current multi-parametric MRI to supplant the need for TRUS guided biopsy in the diagnostic or surveillance setting[53-55].

Functional imaging/Positron Emission Tomography (PET)

PET has advantages over conventional imaging methods because it quantitatively assesses biologic processes *in vivo* and can assess different processes using specific radiotracers. Processes that can be analyzed include glucose and amino acid metabolism, proliferation, blood flow, and biomarker/receptor status (i.e., androgen receptor, PSMA, etc). Most studies, however, have focused on the accumulation of fluorodeoxyglucose (FDG). Functional imaging has not yet added significant value to the management of PCa.

PET scanning with ^{89}Zr-df-IAB2M: ^{89}Zr is an attractive radiometal for use in immuno-PET because of its physical half-life of 78.41 hours, which is well matched to the pharmacokinetics of the minibody. ^{89}Zr is cyclotron produced, has favorable chemistry allowing linkage to Df-IAB2M with high yields, maintaining high radiochemical purity and stability without compromising minibody binding affinity.

Amendment v3 (01/27/17):

PET scanning with ^{68}Ga-PSMA-HBED-CC: A similar opportunity exists to bring clinically important PET tracer(s) into widespread clinical use readily and inexpensively, through the use of generator-produced Gallium-68 (Ga-68), a positron emitting radionuclide with a 68 minutes half-life. Due to the high cost of establishing and maintaining cyclotron and elaborate radiochemistry facilities, generator-produced positron-emitting radionuclides such as Ga-68 have become an attractive alternative to F-18 (Fluorine-18). Ga-68 has come to be a relevant radionuclide for routine clinical examinations, especially in Europe, due to the commercial availability of Germanium (Ge-68)/ Gallium-68 (Ga-68) generators (271 days half-life) that allow obtaining Ga-68 on-site without the need for a cyclotron. The Ge-68 /Ga-68 generator can be used for up to 1 year and the equilibrium between Ga-68 and Ge-68 is rapidly re- established allowing multiple radiotracer preparations in a day from a single generator.

3. PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Patients must have histologically or cytologically confirmed localized prostate cancer that are scheduled to undergo radical prostatectomy.

3.1.2 Age $\geq$18 years.

3.1.3 Patients must have laboratory values consistent with eligibility to undergo a radical prostatectomy:

- creatinine ≤1.5 X upper limit of normal
- creatinine clearance > 60 mL/min

3.1.4 The effects of ^{89}Zr-df-IAB2M on the developing human fetus at the recommended therapeutic dose are unknown. For this reason, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while her male partner is participating in this study, she should inform her treating physician immediately.

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

3.2 Exclusion Criteria

3.2.1 Treatment or plans for treatment with radiation therapy, surgery, chemotherapy, or investigational therapy between the time of conventional imaging, ^{89}Zr-df-IAB2M PET/CT and the surgical resection used for the study evaluation.

3.2.2 Transrectal prostate biopsy performed less than four weeks prior to ^{89}Zr-df-IAB2M administration.

3.2.3 Uncontrolled illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4. REGISTRATION PROCEDURES

Patient Registration

Eligibility will be confirmed as defined in the Section entitled Criteria for Patient/Subject Eligibility. Informed consent will be obtained by following procedures defined in the Section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

5. INVESTIGATIONAL IMAGING PLAN

5.1 Agent administration

^{89}Zr-df-IAB2M:

^{89}Zr-df-IAB2M will be administered under IND No. 130768. ^{89}Zr-df-IAB2M will be administered on an *outpatient* basis. Reported adverse events and potential risks 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 patient's malignancy.

^{68}Ga-PSMA-HBED-CC:

Single intravenous dose of ^{68}Ga-PSMA-HBED-CC (5 ±2 mCi) will be administered under IND # 124495 on an *outpatient* basis. Intravenous access must be well established prior to initiating infusion. The injection will be given by slow intravenous bolus. Following completion of the ^{68}Ga-PSMA-HBED-CC injection, a normal saline flush (approximately 10 mL) will ensure that all ^{68}Ga-PSMA-HBED-CC remaining in the infusion line is injected.

5.2 General concomitant medication and supportive care guidelines

Allergic events will be managed as follows: rash, pruritis, urticaria and wheezing will be treated with diphenhydramine or equivalent H1 blocker and/or steroids as clinically appropriate. Anaphylaxis or anaphylactoid signs or symptoms will be treated with steroids and/or epinephrine as clinically indicated. Infusion-related reactions (fever,

rigors) will be treated with acetaminophen, meperidine or other narcotics and diphenhydramine hydrochloride as clinically appropriate.

5.3 Monitoring vital signs pre/post ^{89}Zr-df-IAB2M and ^{68}Ga-PSMA-HBED-CC

The injection of ^{89}Zr-df-IAB2M 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. Subjects will be offered/receive pre–medication prior to the investigational agent infusion. 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 before the infusion and 30 minutes and 60 minutes after the infusion. If any subject has any adverse reaction at 60 minutes, they will stay longer until it is resolved.

5.4 Duration of study participation and criteria for removal from study

For completion of all scheduled visits and study procedures, a patient may participate for up to 11 weeks or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents administration of ^{89}Zr-df-IAB2M, ^{68}Ga-PSMA-HBED-CC or prostatectomy,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further participation in the judgment of the investigator.

5.5 Duration of Follow Up

Patients will be followed for six to seven weeks after their radical prostatectomy. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6. DOSING DELAYS/DOSE MODIFICATIONS

Not applicable.

7. 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 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.

7.1 ^{89}Zr-df-IAB2M Potential Risks

There are no known contraindications for ^{89}Zr-df-IAB2M. No drug related adverse events were reported in the Phase I study. Because of the potential for infusion or allergic reaction, the subject should be monitored for safety for one hour following the infusion.

The 14 day sterility testing will not be completed at the time of ^{89}Zr-df-IAB2M infusion. The ^{89}Zr-df-IAB2M dose will be released based on the controlled process along with a history of sterile lots. If a sterility test becomes positive any time during the 14-day period, the Investigator will be notified immediately. The subject who received the ^{89}Zr-df-IAB2M will be contacted and requested to have an office visit including blood cultures and, if clinically indicated, started on broad spectrum intravenous antibiotics (Vancomycin and Imipenem).

Radiation Dosimetry: ^{89}Zr and the CT component of the PET/CT study expose patients to ionizing radiation.

In the phase I/IIa study 18 patients received a total of four PET scans. For each PET scan (considered discrete time points) the radioactivity levels in the brain, lungs, heart, liver, gallbladder, kidneys, intestines, urinary bladder and whole body were obtained and recorded. Time activity curves were generated, fit to an exponential model using the OLINDA/EXM software, and the number of disintegrations in the source organs were determined and used to estimate the organ radiation dose. The radiation doses estimates are shown in Table 1.
The lower large intestinal wall received the largest estimated dose (2.0± 0.78mSv/MBq) followed by the liver (1.74± 0.68 mSv/MBq) and the kidneys (1.56± 0.48 mSv/MBq). The Effective Dose was 0.687 ± 0.141 mSv/MBq, thereby resulting in an estimated Effective Dose of 63.5 mSv for the anticipated 2.5 mCi injection in the present trial.

Table 1: Tabular Summary of Subjects

Radiation Dose Estimates (mSv/MBq administered) for Standard Adult Male Phantom:

Target organ	Mean	SD
Adrenals	0.57	0.098
Brain	0.10	0.0087
Breasts	0.24	0.026
Gallbladder Wall	0.84	0.20
LLI Wall	1.99	0.78
Small Intestine	0.69	0.17
Stomach Wall	0.42	0.054
ULI Wall	1.25	0.41
Heart Wall	0.622	0.066
Kidneys	1.56	0.48
Liver	1.74	0.68
Lungs	0.51	0.058
Muscle	0.31	0.035
Ovaries	0.60	0.15
Pancreas	0.53	0.083
Red Marrow	0.35	0.043
Osteogenic Cells	0.38	0.038
Skin	0.20	0.022
Spleen	0.38	0.041
Testes	0.25	0.027
Thymus	0.32	0.028
Thyroid	0.24	0.021
Urinary Blad. Wall	0.36	0.062
Uterus	0.44	0.076
Total Body	0.36	0.048

Target organ	Mean	SD
EDE	0.76	0.14
ED	**0.69**	**0.14**

Amendment v3(01/27/17):

7.2 ^{68}Ga-PSMA-HBED-CC Potential Risks

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

Based on prior studies, the known side effects, risks, and hazards associated with the administration of ^{68}Ga-PSMA-HBED-CC include: infusion reaction (fever, chills, rash, hypotension, and/or hypertension after injection) and transient hepatic enzyme elevations. In addition, allergic reactions including anaphylaxis are a possibility.

Radiation Dosimetry: Radiation dose estimates for ^{68}Ga-PSMA-HBED-CC were provided by Professor Marcus Schwaiger of *Technische Universität München* (*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[56].

Table 2: Radiation dose estimates for Humans administered 148 or 185 MBq (4 or 5 mCi) of 68Ga-PSMA-HBED-CC

Administered dose (Mbq)	**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[57].

7.3 Adverse Event Characteristics

- **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.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.4 Recording of Adverse Events

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

7.5 Serious Adverse Event (SAE) Reporting

7.5.1. Definition of SAE

SERIOUS ADVERSE EVENTS 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.

7.5.2. Reporting of SAE to IRB

All SAEs occurring on this study will be reported to the IRB according to the

IRB policy. The IRB requires immediate reporting of all unexpected and study-related (definite or probable) adverse events. The following procedure will be followed for reporting SAE to the IRB:

- Complete the SAE Cover Sheet (See Appendix B)
- If the event is unexpected AND definitely or probably related to the study, complete the IRB Unexpected, Study-related Adverse Events, Incidents, and Information Reporting Form. This form should be submitted within 24 hours of investigator notification of the event.
- If the event is expected OR possibly or unrelated to the study, only the SAE Cover Sheet must be completed. These events will be reported to the IRB at the time of continuing renewal on the Adverse Event & IND Safety Reporting Cumulative Table.

Forms may also be downloaded from the IRB website at: **http://www.med.cornell.edu/research/for_pol/ins_rev_boa.html**

7.5.3. Reporting of SAE to FDA

If an SAE occurs on this study, the event will be filed on a MedWatch form with the FDA.

CDER-only Biologic INDs:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biologic Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

8. PHARMACEUTICAL INFORMATION

A description of potential risks associated with ^{89}Zr-df-IAB2M can be found in Section 7.1.

8.1 ^{89}Zr-df-IAB2M (IND#:130768)

8.1.1 Radiolabeling of ^{89}Zr-df-IAB2M:

^{89}Zr-df-IAB2M is produced and will be administered under IND No. 130768. For this study, IAB2M is conjugated to desferrioxamine (Df) for labeling with ^{89}Zr. The final investigational drug product is ^{89}Zr-df-IAB2M and is supplied as a sterile filtered solution in a sterile vial sealed with rubber septa and closed with an aluminum stopper. ^{89}Zr is chelated by the Df moiety of the Df-IAB2M conjugate. No materials of animal origin are used in the manufacturing of ^{89}Zr-df-IAB2M. Manufacturing of Df-IAB2M has been performed and

overseen by ImaginAb, Inc. U.S. df-IAB2M is provided by ImaginAb under IND# 118810.

The labeling of df-IAB2M with ^{89}Zr is performed at Weill Cornell Medicine by Shankar Vallabhajosula, Ph.D., Chief of Radiochemistry and the Professor of Radiochemistry/Radiopharmacy in Radiology. df-IAB2M is provided by ImaginAb, under IND# 118810. The process for the manufacture of the ^{89}Zr-df-IAB2M has been submitted and reviewed as a part of the IND #130768. The radiochemistry staff at Weill Cornell Medicine will be responsible for QC testing and batch release of ^{89}Zr-df-IAB2M for clinical use.

The final study drug product is 2.5 (+/- 10%) mCi of ^{89}Zr-df-IAB2M formulated in solution consisting of 5% human serum albumin solution USP, 4mg/mL gentisic acid and 0.2M sodium acetate solution, aseptically mixed with df-IAB2M to achieve a total protein dose of 10 mg.

8.1.2 Radiopharmacy dispensation of ^{89}Zr-df-IAB2M:

The Nuclear Medicine Physician or designee is responsible for ensuring that the delivery of the investigational drug product from the Radiopharmacy to the facility site for patient infusion are correctly labeled, received, handled, and stored in accordance to ICH and applicable government regulations, local/state laws, and used in accordance with this protocol.

The syringe will be shielded by secondary lead containers for radiation protection purposes. The exact radioactive dose administered must be determined by measuring the ^{89} Zr-activity in the syringe before infusion and after infusion by using a dose calibrator, with radioactivity decay correction for infusion time. Measured radioactivity values and times of measurement will be documented, as well as the total injected volume. All unused study drug product must be returned to the Radiopharmacy or destroyed in accordance with institutional policies.

8.1.3 Availability

df-IAB2M is an investigational agent supplied to investigators by ImaginAb. The process for the manufacture of the ^{89}Zr-df-IAB2M by the Radiochemistry/Radiopharmacy Lab in the department of Radiology at Weill Cornell Medicine has been submitted and reviewed as a part of the IND # 130768.

8.1.4 ^{89}Zr-df-IAB2M Accountability

^{89}Zr-df-IAB2M Inventory Records – 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 ImaginAb on a Drug Accountability Record Form (DARF).

8.1.5 ^{89}Zr-df-IAB2M Infusion

^{89}Zr-df-IAB2M will be administered under IND No. 130768. A 10 mg mass dose containing 2.5 mCi ^{89}Zr labeled to 2-3 mg of df-IAB2M and 7-8mg of cold (unlabeled) df-IAB2M will be injected intravenously. The dispensed dose will be infused under the direct supervision of a Nuclear Medicine Physician or designee. Patients will be monitored for safety during and up to one hour after the injection.

Regulatory agencies require accounting for the disposition of all investigational drugs received by each clinical site. Information on drug disposition required by law consists of: 1) date received; 2) date dispensed; 3) quantity dispensed, and 4) patient identification number to whom the drug was dispensed. The Investigator is responsible for accounting for all unused study drug and all used study drug.

8.2 ^{68}Ga-PSMA-HBED-CC (IND #124495):

8.2.1 Radiolabeling of ^{68}Ga-PSMA-HBED-CC:

ABX Advanced Biochemical Compounds, Radeberg, Germany produces PSMA-HBED-CC. Upon purchase and shipment to Weill Cornell Medicine, it will be labeled with ^{68}Ga with final product being ^{68}Ga-PSMA-HBED-CC. Radionuclide conjugation will be done at the Division of Nuclear Medicine, Department of Radiology, Weill Cornell Medicine.

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

8.2.2 Radiopharmacy dispensation of ^{68}Ga-PSMA-HBED-CC:

The Nuclear Medicine Physician or designee is responsible for ensuring that the delivery of the investigational drug product from the Radiopharmacy to the facility site for patient infusion and also to make sure that it is correctly labeled, received, handled, and stored in accordance to ICH and applicable government regulations, local/state laws, and used in accordance with this protocol.

The syringe will be shielded by secondary lead containers for radiation protection purposes. The exact radioactive dose administered must be determined by measuring the ^{68}Ga-activity in the syringe before infusion and after infusion by using a dose calibrator, with radioactivity decay correction for infusion time. Measured radioactivity values and times of measurement will be documented, as well as the total injected volume. All unused study drug product must be returned to the Radiopharmacy or destroyed in accordance with institutional policies.

8.2.3 Availability

PSMA-HBED-CC is an investigational agent supplied to investigators by ABX Advanced Biochemical Compounds, Radeberg, Germany. The process for the manufacture of the ^{68}Ga-PSMA-HBED-CC by the Radiochemistry/Radiopharmacy Lab in the department of Radiology at Weill Cornell Medicine has been submitted and reviewed as a part of the IND # 124495.

8.2.4 ^{68}Ga-PSMA-HBED-CC Accountability

^{68}Ga-PSMA-HBED-CC Inventory Records – 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 ABX Advanced Biochemical Compounds, Radeberg, Germany on a Drug Accountability Record Form (DARF).

8.2.5 ^{68}Ga-PSMA-HBED-CC Infusion

Single intravenous dose of ^{68}Ga-PSMA-HBED-CC (5 ±2 mCi) will be administered under IND # 124495 on an *outpatient* basis. Intravenous access must be well established prior to initiating infusion. The injection will be given by slow intravenous bolus. Following completion of the ^{68}Ga-PSMA-HBED-CC injection, a normal saline flush (approximately 10 mL) will ensure that all ^{68}Ga-PSMA-HBED-CC remaining in the infusion line is injected.

9. IMAGING AND SPECIAL STUDIES

9.1 Imaging and histopathology

9.1.1 3T multi-parametric pelvic MRI

9.1.1.1 Method of Assessment

3T mpMRI preoperative images will be read by GU radiologist reporting: location, size and features of lesions suspicious for PCa. Lesional criteria on MR are defined as being well-circumscribed with low signal intensity on T2 weighted images with associated diffusion restriction on ADC maps. Dynamic contrast enhanced (DCE) MR imaging on preoperative images will also be evaluated subjectively for early and intense enhancement followed by rapid washout, findings that are all associated with prostate cancer. The radiologist will have access to TRUS biopsy results.

An MR /lesional analysis will be performed. Lesional criteria on MR will be defined as well-circumsribed low signal intensity on T2 weighted images with associated diffusion restriction on ADC maps. Dynamic contrast enhanced MR imaging on preoperative images will also be evaluated subjectively for early and intense enhancement followed by rapid washout, findings that are all characteristic of prostate cancer.

9.1.1.2 Timing of Assessment

Will be performed within six weeks prior to or on Visit 3. The investigators will attempt to schedule the mpMR as close to the date of the PET/CT so that the post-TRUS biopsy changes do not confound the differences between the two imaging modalities.

9.1.2 ^{89}Zr-df-IAB2M PET/CT imaging

9.1.2.1 Method of Assessment

PET/CT will be read twice by a nuclear medicine physician. For the first interpretation, the physician will be blinded from clinical history, biopsy results and conventional

imaging modalities. After the initial reading, the nuclear medicine physician will be provided with both the TRUS biopsy results and previously read scans for the repeat PET/CT reading. During each interpretation, the physician will record all abnormal, suspicious areas with higher uptake than adjacent or contralateral background. Each abnormal area will be recorded on a map of the prostate along with maximum standardized uptake value (SUV_{max}) and mean standardized uptake value (SUV_{mean}).

Subsequently, the accumulation of ^{89}Zr-df-IAB2M will be compared to areas of known PCa foci on histopathology and mpMRI, as described in Section 13. After completion of the histopathology, the PET/CT findings will be compared to histopathology to perform a lesion by lesion analysis with histopathology serving as the reference gold standard. This analysis will primarily include tumors ≥ 0.5 cm^3 on histopathology, with a secondary assessment of any lesions <0.5 cm^3. Similarly, areas of increased uptake identified on IAB2M, which had been read as suspicious for cancer will be compared to histopathology to determine the underlying pathology (e.g., inflammation, benign prostatic hyperplasia).

9.1.2.2 Timing of Assessment

Infusion of ^{89}Zr-df-IAB2M will occur during Visit 2. Imaging will be performed 2-3 days after the injection on Visit 3. Please refer to the Calendar of Events (Section10).

9.1.3 ^{68}Ga-PSMA-HBED-CC PET/CT imaging

Patient preparation should be according to the policies and procedures of the local imaging site (CIBC). 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 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. The subject will be scheduled for the injection and imaging between screening and prostatectomy depending on subjects' availability and compliance.

9.1.4 Histopathology

9.1.4.1 Method of Assessment

All patients will undergo radical prostatectomy and potential lymph node dissection. The prostate gland will be fixed in formalin for 12-24 hours at room temperature. After fixation, the gland will be processed according to standard institutional protocol, as follows:

1) Weigh the prostate gland with attached seminal vesicles and vasa deferentia

2) Measure the prostate, seminal vesicles, and vasa deferentia

3) Orient the gland. Note and describe any nodularities or asymmetry to the gland; tissue disruptions; defects; or surface irregularities; adherent tissues/organs, etc.

4) Ink the entire specimen—green on the right side and black on the left.

5) Amputate both seminal vesicles and vasa deferentia at their attachment to the prostate and submit the most proximal portion of each for histology (i.e., submit the portion of SV/VD nearest their juncture with the prostate).

6) Amputate the most distal (apical) 5-7 mm of the gland. Place the broad cut surface of this "cone" of tissue onto the table and section perpendicularly to the midline (into ~3 mm strips). Submit the strips on "edge" for margin (ink) evaluation. When submitting, if the strips are not too long, leave the left and right sides connected and submit the anterior and posterior halves separately (e.g., A2-anterior apex, A3-posterior apex). If the strips are too long to fit into a cassette, divide the strips into left and right halves. Maintain the separation of anterior and posterior halves and designate appropriately in the summary of sections (e.g., A2-left anterior apex, A3-right anterior apex, A4-left posterior apex, A5- right posterior apex).

7) Amputate the most proximal (basal) 5-7 mm of the gland, which should include the bladder neck margin, and process this "cone" of tissue in the same manner as the apex (Step 6).

8) Section the remaining prostate perpendicular to the urethra into 4 mm thick slices, starting at the apex and continuing towards the base until completely sectioned. Designate the slices/levels as A, B, C, etc., starting from the apex. Generally, an average 35 gram prostate should be sectioned into at least five levels (A to E).

Sections to submit for histology: Right and left seminal vesicle and vas deferens (1-2 cassettes total, depending on size); Bladder neck/base margin (2-4 cassettes, divided as above); Apical margin (2-4 cassettes, as above)

Remaining prostate will be submitted as follows:
Each slice/level is quadrisected into 1) left anterior 2) left posterior 3) right anterior and 4) right posterior, as with the apex and base margins. However, DO NOT cut the slices/levels into strips; submit as intact quadrant pieces and designate in the summary of sections (e.g., left anterior slice A should be "LAA", and left posterior slice A should be "LPA" and so forth).

A pathologist, blinded to the imaging results, will independently review all slides. Cases will be reported according to standard pathologic procedures including the presence of tumor, Gleason score, extracapsular extension, and seminal vesicle invasion. In addition, using an anatomical map of the prostate, each individual tumor

focus will be outlined and annotated (location within the gland, tumor size, tumor stage, and Gleason score). Any other abnormal areas/findings within the specimen will also be annotated. Immunohistochemical assessment of PSMA expression and/or neuroendocrine features will be performed as specified in source documentation. A tabular list of all cancer lesions will be made for each patient based on size and/or stage of each tumor and listing Gleason score, stage, and location. This list will be used to review and correlate with imaging findings on IAB2M PET/CT and mpMRI.

9.1.3.2 Timing of Assessment

Histopathologic specimen assessment will occur after Visit 4, radical prostatectomy. Please refer to the Calendar of Events (Section10).

9.2 **Special Studies**

9.2.1 Antigenicity Testing (HAHA)

9.2.1.1 Method of Assessment

5 mL of whole blood will be drawn by peripheral venipuncture in a yellow top STT tube (5 mL) for HAHA evaluation.

9.2.1.2 Timing of Assessment

Blood draws for HAHA determinations will be obtained on Visit 2, prior to ^{89}Zr-df-IAB2M infusion and at Visit 5 (refer to Section 10).

10. STUDY CALENDAR

Table 3: Schedule of Assessments

Protocol Assessments	Screening[a] Visit 1	Infusion Visit 2	Imaging Visit 3	Prostatectomy Visit 4	HAHA Visit 5
	Day -28 to -1	Day 0	Day 2 - 4	Within 3-30 days after visit 3 (preferably 1 week after scan)	6 Weeks ±1 week after Visit 4
Informed consent	**X**				
Demographics	**X**				
Medical history	**X**	**X**			
Concomitant medications[b]	**X**	**X**	**X**		
Physical exam	**X**	**X[c]**			
Karnofsky Performance	**X**				
Vital signs[c]	**X**	**X**	**X**		
Blood lab samples[d]	**X**	**X**	**X**		
Radiographic studies[e]	**X**	**X[e]**	**X[e]**	**X[e]**	
HAHA Blood		**X[f]**			**X[f]**
^{89}Zr-df-IAB2M tracer administration		**X**			
^{68}Ga-PSMA-HBED-CC administration and PET/CT scan		**X[g]**			
^{89}Zr-df-IAB2M PET/CT scan			**X**		
Adverse events		**X**	**X**		

[a] Screening evaluation should be performed following informed consent and within 4 weeks before enrollment, except when noted otherwise. Evaluations performed as standard of care before informed consent but within the 4-week window need not be repeated.

[b] Concomitant medication refers to medication received by subjects from the point of signed informed consent through the end of Visit 6.

[c] Vital signs include height, weight, temperature, blood pressure and heart rate. Height and weight are obtained only at Visit 1. During the Infusion Visit 2, serial vital signs will be monitored pre-infusion, then 30 minutes

and 60 minutes post-infusion (±5 minutes).

[d] Laboratory samples collected include Chemistry panel, CBC (hematology), PSA (first blood draw only), and serum testosterone level (first blood draw only). All Screening laboratory samples are to be collected within 14 days before enrollment or at Visit 2, Pre-infusion (serum testosterone level may be up to **4** weeks before enrollment or Visit 2, prior to ^{89}Zr-df-IAB2M infusion) and again at Visit 3.

[e] Radiographic evaluations include a modified (3T multiparametric) MRI of the pelvis, if clinically indicated. Radiographic studies are to be performed within 6 weeks before, or on Visit 3/Day 2, provided the patient has not received a new intervening anti-cancer treatment.

[f] Blood sample will be drawn via venipuncture for HAHA evaluation pre-infusion at Visit 3and repeat samples will be taken at Visit 5 (week 6, +/- 1 week).

[g] ^{68}Ga-PSMA-HBED-CC administration followed by PET/CT scan 1 – 3 hours later must be done prior to ^{89}Zr-df-IAB2M tracer administration on Visit 2 otherwise the 68Ga-PSMA-HBED-CC administration and PET/CT scan can be done anytime between screening and prostatectomy visit.

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, and efficacy data for all enrolled patients.

11.1.1 REDCap

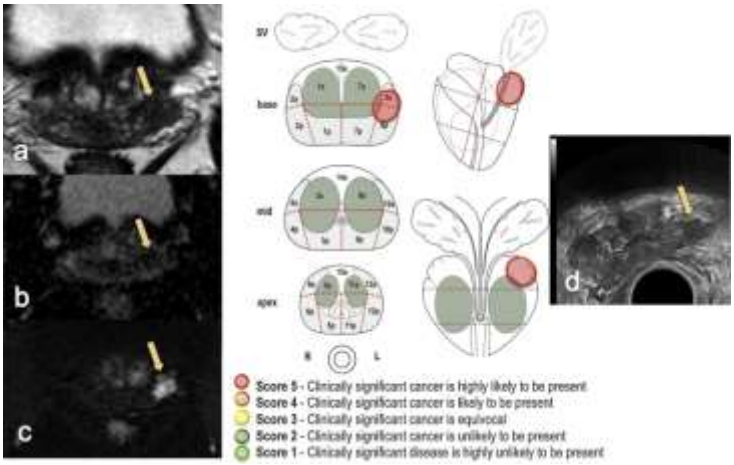
REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill-Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array 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.

12. STATISTICAL CONSIDERATIONS

Data analysis and image interpretation will be performed in a manner similar to the strategy employed by Osborne et al. [58] and Turkbey B et al[59], with both a lesion specific and sector-specific analysis. For the sector-analysis, the prostate gland will be divided into segments as described in the START criteria[58]. A lesion-based analysis will also be performed after review of histopathology.



Sectors of the prostate gland as described by the START criteria, Moore C, et al.

12.1 Study Design/Endpoints

The present study is a phase II, open label, single-center, non-randomized, single-dose study.

Primary endpoints

The primary endpoint of this study is the proportion of subjects with PSMA-positive (identified by H&E staining and immunohistochemistry) "dominant" PC lesion(s) greater than 5mm in diameter, whose lesion(s) have been successfully identified by ^{89}Zr-df-IAB2M imaging. Because this is an exploratory pilot study, no formal sample size/power calculation is required. However, with a sample size of 20 patients in the study, a two-sided 95% confidence interval for the proportion of patients successfully imaged by ^{89}Zr-df-IAB2M can be constructed to be within ± 19.0% of the observed proportion of patients with successful imaging by ^{89}Zr-df-IAB2M. This calculation assumes an ^{89}Zr-df-IAB2M imaging-success proportion of 75%. All estimates from the study will serve as preliminary data (i.e., hypothesis-generating) for future studies.

- Descriptive statistics will be calculated for the study cohort:
  - Median and inter-quartile range or mean and standard deviation for age, biopsy Gleason score (1^o, 2^o, Total), number of cores on biopsy, number of positive cores on biopsy, PSA, number of lesions on prostatectomy pathology (RP pathology), lesion size on RP pathology, Gleason score (1^o, 2^o, and total) on RP pathology.
- Lesion-based analysis:

- SUV_{max} measurements of ^{89}Zr-df-IAB2M PET/CT images obtained in tumor and non-tumor regions will be compared. To account for intrapatient correlation of multiple lesions, a linear mixed-effects model with a random intercept will be used to estimate and compare the mean SUV_{max} values in different regions. $P<0.05$ will be indicative of a significant difference as described by Turkbey B, et al.[59]

- Positive and negative predictive values will be estimated by using generalized estimating equations (GEE) modeling, with logit link function and a working independence correlation structure. Robust variance estimation and the delta method will be used to calculate standard errors of the positive and negative predictive values. The Wald test with robust variance estimates will be used for statistical inference. Subgroup analyses will be performed for high, moderate, and low risk disease (see secondary endpoints).

- Receiver operating curve characteristics (ROC) will be constructed to determine the predictive ability of SUV level on detection of any disease and disease severity as correlated to histopathology.

Secondary endpoints

- To determine the ability of *in vivo* ^{89}Zr-df-IAb2M PET/CT to correlate with pathological features (i.e. Gleason pattern, diameter and PSMA-positivity):

    - The Wilcoxon rank sum test will be used to compare lesions that were and were not identified on *in vivo* PET with respect to lesion size, immunohistochemical PSMA staining, and Gleason score (demonstrated by histopathology). This lesion based analysis will be performed in a similar fashion as described above.

- To compare the *in vivo* ^{89}Zr-df-IAb2M PET/CT and ^{68}Ga-PSMA-HBED-CC PET/CT to *in vivo* mpMRI:

    - The diagnostic accuracy of ^{89}Zr-df-IAB2M PET/CT and ^{68}Ga-PSMA-HBED-CC PET/CT imaging in patients with prostate cancer will be compared with that of mpMRI imaging by using sector-based analysis, as described by Turkbey B et al.[59] The estimates of patient-specific sensitivity and specificity will be defined as the proportion of true-positive sectors and the proportion of true-negative sectors in each patient. Overall sensitivity and specificity will then be estimated by calculating the mean (and standard error) of these individual-

specific estimates across patients. Differences in sensitivity and specificity between modalities will then be evaluated by the Wilcoxon signed-rank test.

- To determine the sensitivity, specificity, PPV, NPV of IAB2M to detect all pathologically identified PCa lesions that do not meet criteria for clinical significance (defined as: < 0.5 cm3 with Gleason pattern < 4):
    - As above, but for lesions considered not to be clinically significant.
- To correlate IAB2M positivity (SUV) and lesion size:
    - The Spearman-rank correlation coefficient will be used to evaluate the association between SUV and size of the lesion (procedure for size measurements are defined in Section 9.1.3).
- To correlate IAB2M positivity (SUV) and Gleason pattern (3, 4 and 5):
    - The Spearman-rank correlation coefficient will be used to evaluate the association between correlate SUV and the histopathologically defined Gleason pattern. The Wilcoxon rank-sum test will also be used to compare SUV_{max} measurements between lesions with low Gleason score (3+4 or lower) and high Gleason score (4+3 or higher).
- To correlate IAB2M positivity (SUV) and PSA:
    - The Spearman-rank correlation coefficient will be used to evaluate the association between SUV and serum PSA levels obtained during screening.
- To determine the ability of *in vivo* ^{89}Zr-df-IAB2M PET/CT to identify extra-prostatic extension:
    - The Wilcoxon rank-sum test will be used to compare SUV_{max} measurements of IAB2M-positive extra-prostatic sites between lesions with and without histopathologically confirmed extra-prostatic extension.
- To determine the ability of *in vivo* ^{89}Zr-df-IAB2M PET/CT to identify occult lymph node involvement:

- The Wilcoxon rank-sum test will be used to compare SUV_{max} measurements of IAb2M-positive lymph nodes between patients with and without histopathologically confirmed lymph node involvement.

- To further evaluate the safety of ^{89}ZR-DF-IAB2M PET/CT and ^{68}Ga-PSMA-HBED-CC imaging.

  - See section on Risks

  Additionally, we will assess whether there is improved sensitivity in detecting localized PCa by combining imaging results. This assessment will be done by estimating the PPV with different combinations of imaging modalities. The incremental effect of an additional imaging modality on PPV will be tested with the Wald test, as described by Turkbey B, et al.[59]

  All p-values will be two-sided with statistical significance evaluated at the 0.05 alpha level. All analyses will be performed in SAS Version 9.4 (SAS Institute, Inc., Cary, NC) and Stata Version 13.0 (StataCorp, College Station, TX).

12.2 Sample Size/Accrual Rate

The sample size for this study will be 20 patients. We anticipate an accrual rate of at least two patients per month. With a sample size of 20 patients in the study, a two-sided 95% confidence interval for the proportion of patients successfully imaged by ^{89}Zr-df-IAB2M can be constructed to be within ± 19.0% of the observed proportion of patients with successful imaging by ^{89}Zr-df-IAB2M. This calculation assumes an ^{89}Zr-df-IAB2M imaging-success proportion of 75%. All estimates from the study will serve as preliminary data (i.e., hypothesis-generating) for future studies."

12.3 Stratification Factors

No stratification factors. However, an interim analysis and efficacy determination based on the primary endpoint will be performed after the first 10 patients have completed Visit 4 and subsequent histopathological analysis has been performed on the prostate specimen.

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APPENDIX A

Performance Status Criteria

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

APPENDIX B

WCMC IRB SAE Reporting Forms

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