^{68}GA-PSMA FUSION PET/MRI FOR IMAGE-GUIDED PROSTATE BIOPSIES

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1 SYNOPSIS

Title	**^{68}GA-PSMA FUSION PET/MRI FOR IMAGE-GUIDED PROSTATE BIOPSIES**
Principle Investigator	Morand Piert, MD
Study Center	Michigan Medicine
Primary Objective(s)	To determine the sensitivity and specificity of mpMRI alone, and mpMRI in combination with ^{68}Ga-PSMA PET (i.e., fusion PET/MRI), for the detection of primary Gleason ≥3+4 PCa. Our primary outcome will be to determine whether the positive likelihood ratio (LR) for ^{68}Ga-PSMA PET-MRI is at least 1.28 greater than that of standard-of-care mpMRI.
Secondary Objective(s)	(1) Safety (2) Frequency of extraprostatic extension, lympho-nodal and/or other metastatic disease positive on ^{68}Ga-PSMA and not identified by conventional imaging
Exploratory Objective	Exploratory assessment of radiomics texture analyses of mpMRI and ^{68}Ga-PSMA PET identified lesions
Test drug(s)	
Name of active ingredient	^{68}Ga-PSMA
Dose	3.0 – 7.0 mCi
Route of administration	Intravenous
Duration of treatment	Once
Indication	Prostate cancer
Diagnosis and main criteria for inclusion	Subjects must fulfill the following criteria: Males (≥ 18 years of age) with prior mpMRI resulting in at least one PI-RADS 3-5 lesion with clinical need for a prostate biopsy procedure can participate, if one of the 2 criteria apply: (1) Known Gleason 6 or 7 prostate cancer undergoing active surveillance or in the confirmation phase of active surveillance, **or** (2) Rising PSA with either a) no prior biopsy or b) single or multiple negative prior biopsies.
Exclusion criteria	Any criterion will result in ineligibility: (1) Prior prostate biopsy within 12 weeks prior to enrollment (2) Acute prostatitis within the last 6 months (3) Current non-urologic bacterial infection requiring active treatment with antibiotics (4) Active other malignancy (except basal cell or squamous cell skin cancer) within the last 2 years (5) Body weight greater than 350 lbs (158 Kg) (6) Inability or unwillingness to receive a prostate biopsy procedure (7) Unable to lie flat, still, or tolerate a PET/CT scan (8) Unable to provide own consent (9) Concurrent severe and/or uncontrolled and/or unstable medical disease other than prostate cancer (e.g. poorly controlled diabetes, congestive heart failure, myocardial infarction within 6 months prior to study participation, unstable and uncontrolled hypertension, acute renal failure of any intensity, chronic renal or hepatic disease, severe pulmonary disease) (10) Prisoner
Study phase	Phase 2
Study design	Open label, single-center, single-dose, diagnostic imaging efficacy study

Methodology	Participating subjects already underwent a clinically indicated prostate MRI resulting in at least one PI-RADS 3-5 lesion performed within 3 months prior to recruitment. Research imaging: ^{68}Ga-PSMA PET/CT. Fusion of mpMRI and ^{68}Ga-PSMA PET and biopsy target selection from mpMRI and ^{68}Ga-PSMA PET. Prostate biopsy procedure (clinically indicated): standard biopsy and additional targeted biopsies to obtain pathological proof. Biopsy targets are selected from mpMRI and ^{68}Ga-PSMA PET.
Type of control	Not applicable
Number of subjects	100
Safety Assessments	Patient vital signs will be taken before and after the administration of the radiopharmaceutical. The patients will also be asked to report adverse events.
Plan for statistical analysis	Visual and semi-quantitative assessment of PET/CT images: Descriptive statistics, frequency tables. We employ mixed effects logistic regression model to estimate the positive likelihood ratio (LR) of the combination of ^{68}Ga-PSMA-guided plus mpMRI-guided biopsy (fusion PET/MRI) for the detection of primary Gleason ≥ 3+4 cancer is higher than for mpMRI-guided biopsy (using PI-RADS version 2) alone.

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2.1 Overall Table of Contents

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

^{68}Ga-PSMA	Gallium-68 labelled prostate-specific membrane antigen (PSMA-HBED-CC, or PSMA-11)
ADL	Activities of Daily Living
AE	Adverse event
ALARA	As low as reasonably achievable
AS	Active surveillance
BP	Blood pressure
BPH	Benign prostate hyperplasia
CRF	Case report form
CRPC	Castrate resistant prostate cancer
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DICOM	Digital imaging and communications in medicine
FWHM	Full width at half maximum
GC	Gas chromatography
GCP	Good clinical practice
HPLC	High-performance liquid chromatography
i.v.	Intravenous
IHC	Immunohistochemistry
INR	International normalized ratio
IRB	Internal review board
LC	liquid chromatography
MRI	Magnetic resonance imaging
mpMRI	Multi-parameter MRI
OSEM	ordered-subset expectation maximization
p.i.	post injection
PCa	Prostate cancer
PET	Positron emission tomography
PET/CT	Hybrid PET with CT
PET/MRI	Positron emission tomography with magnetic resonance imaging
PI	Principal investigator
PI-RADS	Prostate Imaging Reporting and Data System
pPCa	Primary prostate cancer
PSA	Prostate-specific antigen
ROC	Receiver operating characteristic
SAE	Serious adverse event
SD	Standard deviation
SID	Stable isotope dilution
SUV	Standardized uptake value
SOG	Study operation guide
TBR	Tumor-to-background ratio
TRUS	Transrectal ultrasound
VOI	Volume of interest

List of Definitions

Mass dose	Amount of ^{68}Ga- labeled drug substance (“hot” fraction of the active compound, ^{68}Ga-PSMA), plus non-radioactive drug substance, expressed in µg
Radioactive dose	Amount of active ^{68}Ga- labeled drug substance (“hot” fraction of the active compound, ^{68}Ga-PSMA), expressed in MBq
Impurities	Everything not considered in the Mass dose, e.g. by-products, degradation products, radiolysis products
Total quantity	Mass dose plus impurities (for a study following the microdosing concept the total quantity must remain ≤ 100 µg/human)

4 BACKGROUND INFORMATION

4.1 Introduction

Primary prostate cancer (PCa) represents a broad spectrum of disease, ranging from indolent to aggressive and death-causing illness. The highly heterogeneous nature of PCa provides a significant challenge for clinical disease management.[1,2] Organ-confined PCa is an overdiagnosed and overtreated disease, which may result in life-changing side effects.[3] This overtreatment is in part related to the inability to accurately identify PCa with elevated risk. Standard of care management of rising prostate-specific antigen (PSA) levels followed by standard systematic (non-targeted) prostate biopsies is unsatisfactory and leads to the detection of low grade prostate cancer at high rate while - at the same time - missing many significant (Gleason score ≥ 3+4) cancers.[4] The positive predictive value of the standard prostate biopsy is only approximately 30% at a PSA level > 4.0 ng/mL.[5] Accordingly, the recommendation by the United States Preventive Services Taskforce (USPSTF) against PSA screening in 2012 has led to a reduction of the number of PSA tests and prostate biopsies being performed.[6] While this will likely reduce the costs associated with low-risk PCa, it may inadvertently cause a reverse stage migration to more men presenting with advanced disease. It was estimated that omitting PSA screening may lead to a doubling of metastatic disease rates as early as 2025, which would generate many avoidable cancer deaths.[7]

Clearly, a highly accurate, ideally non-invasive, precision imaging test to differentiate low-risk disease from intermediate/high-risk PCa is needed. Accurate imaging allows subsequent tissue verification (via targeted biopsy) of suspicious lesions. Multiparametric magnetic resonance imaging (mpMRI) at 3 Tesla — due to its ability to detect clinically significant PCa and minimize detection of clinically insignificant disease — has been advocated for this task.[8] In fact, mpMRI applying the Prostate Imaging Reporting and Data System (PI-RADS version 2) scoring criteria[9] have demonstrated rising detection rates of significant PCa with increasing PI-RADS scores when combined with targeted prostate biopsies. Image-guided (targeted) prostate biopsies based on mpMRI clearly outperform non-targeted biopsies.[10,11] However, the performance of this approach remains suboptimal for the task of identification of significant disease with high precision.[12,13] In the recent multicenter paired-cohort PROMIS trial of 740 men, the sensitivity of mpMRI for clinically significant prostate cancer was 93%, but the specificity was only 41%, indicating a high false positive rate.[11] When mpMRI is compared to whole-gland pathology from prostatectomy specimens, results are less encouraging. Combining 2 recent studies with a total of 275 subjects (performed at highly experienced institutions), the sensitivity for identification of any Gleason ≥ 7 PCa was below 80%, while at the same time a significant number of low-risk cancers were falsely “overstaged” (specificity 54 – 68 %).[12,14] Furthermore, interpretation of mpMRI, even when using standardized reporting methods (PI-RADS), is difficult and requires considerable training. Among highly experienced prostate MRI specialists, inter-observer variability using PI-RADS classifiers remains too high (κ= ~ 0.5-0.6).[15]

Optimizing the performance of mpMRI—in particular, improving the specificity—is important. We recently published an interim analysis on 36 subjects with 52 mpMRI-identified lesions (Likert scale: low, intermediate, high risk) that showed the addition of ^{18}F-choline PET/CT to mpMRI significantly improves the identification of Gleason ≥ 3+4 PCa over mpMRI alone.[16] Pathology was obtained from mpMRI-guided plus 12-sample standard biopsies. Our unpublished final results obtained from 56 subjects with 90 mpMRI-identified lesions (40 low, 30 intermediate, 20 high risk) indicate that ^{18}F-choline significantly improved the differentiation between clinically significant PCa (Gleason ≥ 3+4) and low-risk or benign disease in men assigned an intermediate-risk score by mpMRI. Furthermore, we were able to demonstrate that the combination of ^{18}F-

choline PET with mpMRI was cost-effective (respective manuscripts have been submitted for publication in "Radiology").

While these results demonstrate that precision imaging utilizing a combination of molecular (PET) data with mpMRI is can be superior to mpMRI alone, the chosen radiotracer (^{18}F-choline, as ^{18}F-choline PET/CT) is ultimately not sufficient for the diagnosis of primary PCa because elevated choline uptake is commonly seen in benign prostatic hyperplasia (BPH) nodules.[17] Under these circumstances, future approval for ^{18}F-choline by the U.S. Food and Drug Administration (FDA) for initial staging of PCa is highly unlikely and alternative strategies are needed.

4.1.1 Significance of PSMA and preliminary data for PET imaging with ^{68}Ga-PSMA

In recent years, ^{68}Ga-labeled ligands to PSMA have shown great promise for PET imaging of recurrent PCa. Particularly important is the fact that radiolabeled small molecules binding to PSMA are capable of identifying PCa at very low PSA levels, opening the door for early targeted treatment of disease recurrence.[18-22] There is also substantial evidence that ^{68}Ga-PSMA outperforms ^{11}C/^{18}F-choline for this indication.[23-26] In contrast to ^{111}In-capromab-pendetide (ProstaScint), these ligands bind to the extracellular domain of this transmembrane glycoprotein and are internalized due to the enzymatic activity of PSMA, which leads to trapping of diagnostic (and therapeutic) radionuclides based on PSMA expression.

PSMA is expressed physiologically in the prostate at low levels as a cell surface protein, and is overexpressed in PCa, particularly in metastatic castration-resistant PCa. In fact, there is growing evidence that PSMA expression increases as a function of tumor aggressiveness and Gleason scores,[27,28] which is a highly useful feature for the differentiation of significant from indolent primary PCa. Accordingly, ^{68}Ga-PSMA provides excellent target-to-background contrast suitable for PET/CT imaging of PCa.[18,29]

While data are still limited, several retrospective reports indicate utility of ^{68}Ga-PSMA PET/CT to diagnose and localize primary PCa. [19,26,30,31] In a small series with 20 patients receiving ^{11}C-choline and ^{68}Ga-PSMA PET/CT, both tracers identified all biopsy-proven significant primary PCa, but ^{68}Ga-PSMA lesion uptake was significantly higher than that of ^{11}C-choline, facilitating lesion detection.[26] Most importantly, ^{68}Ga-PSMA standardized uptake values (SUV) increase with Gleason grading, an important prerequisite for the identification of significant disease. In fact, a drastic increase in ^{68}Ga-PSMA uptake is found for Gleason ≥ 3+4 PCa relative to low-grade disease and benign prostate, with little overlap between groups.[32] In addition, ^{68}Ga-PSMA also appears to have utility for lymph node staging.[33] Based on our pilot data (**Figure 1**) and as demonstrated by Eiber et al.,[19] hybrid or fusion ^{68}Ga-PSMA PET/MRI seems to be particularly useful for the localization and staging of primary disease within the prostate gland with a precision necessary for successful image-guided targeting at biopsy. The current literature regarding this topic is summarized in Bailey et al.[34]

The immunohistochemical (IHC) expression of PSMA has been linked to aggressive features in localized (primary) PCa. PSMA overexpression has been associated with a higher rate of local recurrence after prostatectomy, [35] further substantiating a potential link of ^{68}Ga-PSMA uptake and aggressiveness. This is an important requisite of ^{68}Ga-PSMA when considering patient management within an active surveillance population, particularly since PSA and PSA kinetics are insufficient predictors of aggressive disease.[36] In addition, PSMA is also a promising target for PCa-specific radionuclide therapies.[37]

^{68}Ga-PSMA is not completely specific for PCa, as IHC PSMA expression has been found in the neo-

vasculature of regenerative and reparative conditions.[38,39] Also, recent case reports indicate elevated ^{68}Ga-PSMA uptake in pulmonary infections (tuberculosis), non-prostatic malignancies (lung cancer, lymphoma),[40,41] benign neoplasms,[42] and trauma. Nonetheless, in Figure 1, we demonstrate the utility of ^{68}Ga-PSMA for the distinction of cancer and inflammation in the prostate. While both mpMRI scans in the figure were reported prospectively as high risk for aggressive PCa (PI-RADS 5), elevated ^{68}Ga-PSMA uptake was seen only in PCa, but not in the site(s) of granulomatous inflammation.

Though the vast majority of aggressive prostate cancers express PSMA and display increasing IHC staining intensities with increasing Gleason scores,[28] a small subset of aggressive PCa appears to lack PSMA expression. Accordingly, the potential for absent ^{68}Ga-PSMA uptake in aggressive primary PCa needs to be considered.

4.1.2 Radiomics

Traditionally, medical images are treated as pictures intended solely for visual interpretation, which undercuts their information rich nature as mineable data.[43] Most human cancers exhibit strong molecular, morphological and phenotypic differences that are not easily assessable with standard image analysis techniques.[44] Comprehensive quantification of imaging features, called radiomics, can extract such image metrics (intensity, shape, texture, etc.) into mathematical classifiers of the tumor diagnostic or prognostic phenotype from single or hybrid anatomical and functional images.[45-50] An example is shown in **Figure 1**. This is particularly useful in case of PCa, which often presents as heterogeneous multifocal disease of varying clinical significance.[51] Fehr *et al.* showed that texture features from mpMRI could achieve good classification of Gleason patterns.[52] Radiomics not only serves classification purposes, but can also aid in understanding the underlying biology of the observed imaging phenotype and the risk variations among the different foci of the disease, subsequently informing clinical staging and effective image-guided therapeutic intervention in PCa.[53]

Since the utility of radiomics analyses are unknown for the combination of ^{68}Ga-PSMA and mpMRI, we will retrospectively assess its value to identify significant primary PCa.

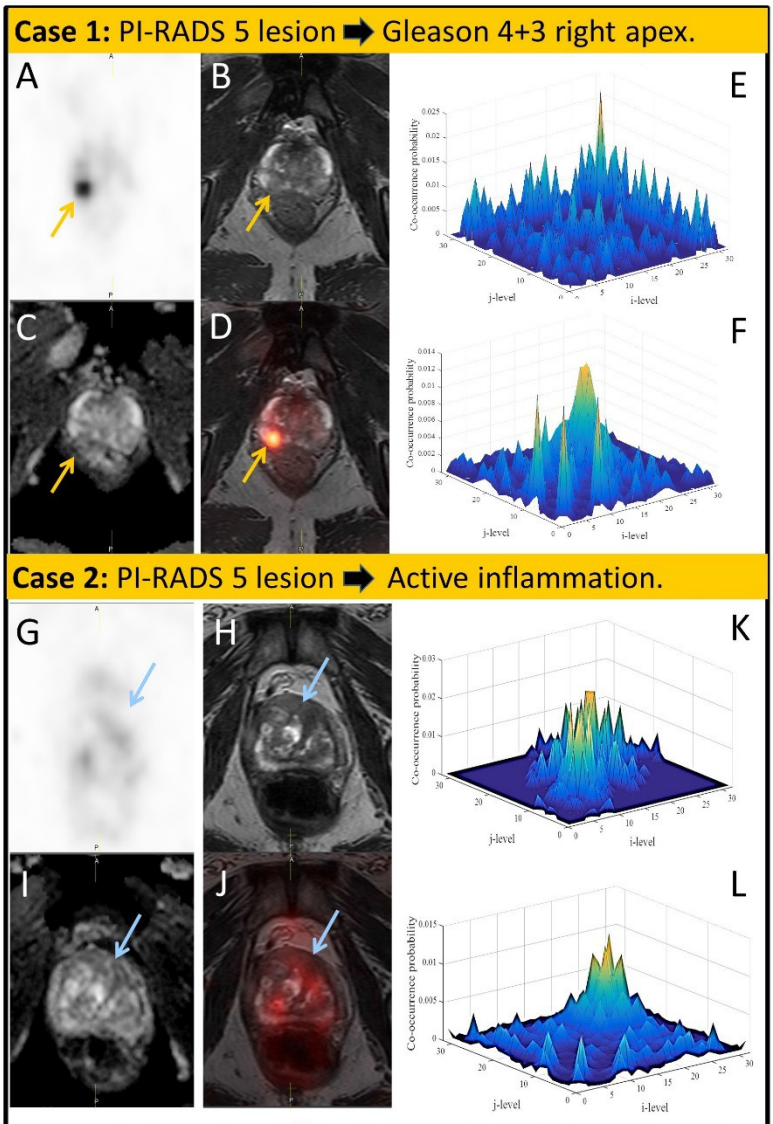

Figure 1. Registered axial ^{68}Ga-PSMA PET (A,G; SUV range 0 – 10 for both scans), T2W MRI (B,H), ADC map (C,I), PET/MRI (D,J), and radiomics texture map from PET (E,K) and ADC (F,L) of two subjects for which a mpMRI resulted in a PI-RADS 5 lesion. **Upper panel (A-F), case 1:** Right apical Gleason 4+3 PCa (orange arrows), identified at targeted biopsy with intense focal ^{68}Ga-PSMA uptake (SUV median 7.0). The ^{68}Ga-PSMAtexture map (E) shows a high degree of heterogeneity within the tumor. **Lower panel (G-L), case 2:** targeted biopsy resulted in active granulomatous inflammation (blue arrows) without elevated ^{68}Ga-PSMA uptake (SUV median 1.4). Texture map from ^{68}Ga-PSMA PET showed low heterogeneity, while texture maps from ADC are very similar for both cases (F, L).

5 STUDY OBJECTIVES AND PURPOSE

The primary study aim is the prospective comparison of standard-of-care mpMRI vs. combined ^{68}Ga-PSMA PET and mpMRI (^{68}Ga-PSMA fusion PET/MRI) for the detection and risk-stratification of clinically significant primary PCa.

Following (clinical) mpMRI and (research) ^{68}Ga-PSMA PET/CT imaging, we will perform targeted and standard 12-sample prostate biopsies. Biopsy targets will be defined from mpMRI and ^{68}Ga-PSMA PET; mpMRI interpretation will be done in accordance with PI-RADS v2.

Primary objective: We will determine the sensitivity and specificity of mpMRI alone and mpMRI in combination with ^{68}Ga-PSMA PET (i.e., fusion PET/MRI) for the detection of primary Gleason ≥3+4 PCa. Primary outcome measure: determination whether the positive likelihood ratio (LR) for ^{68}Ga-PSMA PET-MRI is at least 1.28 greater than that of standard-of-care mpMRI. Reference standard will be pathology defined as targeted plus standard prostate biopsy, and prostatectomy, when available.

Secondary objective: We will determine the safety of ^{68}Ga-PSMA. We will also determine frequency and location of ^{68}Ga-PSMA positive lesions, such as potential extra-prostatic extension, lympho-nodal and/or distant metastatic disease positive on ^{68}Ga-PSMA but not identified by conventional (standard) imaging. While histological verification would be desirable, the decision for or against tissue verification will depend on clinical decisions making only, and not be required due to study participation.

Exploratory objective: We will evaluate whether radiomics classifiers (obtained from mpMRI and ^{68}Ga-PSMA identified targets) could improve identification and risk stratification of significant PCa. Reference standard will be pathology defined as targeted plus standard prostate biopsy, and prostatectomy, when available.

6 OVERALL STUDY DESIGN AND PLAN

6.1 Study rationale

6.1.1 Rationale for administered radioactivity doses

For ^{68}Ga-PSMA, we will use the administered radioactivity dose ranges described in the literature. The administered dose may vary between 110 – 370 MBq (equivalent range of 3.0 – 7.0 mCi. The amount of radioactivity per subject is in accordance with the radiation protection principle “as low as reasonably achievable (ALARA)”, whilst ensuring image quality that is suitable for the planned analyses [18,20].

6.2 Study timeframe

The recruitment period for the study is expected to last up to 4 years. The duration of patient participation in this study will end with the clinically indicated biopsy procedure.

6.3 Risks and benefits to subjects

There are no expected direct benefits to subjects who participate in this trial. However, participants may benefit under certain conditions. First, if targeted biopsies identify a significant prostate cancer lesion not identified on standard biopsies or MRI, the patient would potentially benefit from the detection of such disease. Second, patients who will undergo subsequent definitive treatment may benefit if imaging identifies aggressive features such as extraprostatic spread or nodal metastases that otherwise would not have been identified. Also, the treatment team will be notified about the study imaging results in case of any other unexpected relevant abnormal findings. Accordingly, treatment decisions can be made on the basis of ^{68}Ga-PSMA scan results.

The risks to subjects mainly relate to the procedures employed for i.v. injection of the investigational radiotracers. Also, there are risks due to the placement of an i.v. catheter for drug injections as intravenous injections as well as small amounts of blood withdrawn. These are known to carry a small risk of infection and hematoma (infrequently occurring). There is also a small increase in risk due to additional prostate biopsy cores obtained with the clinically indicated prostate biopsy procedure. This risk is limited as we will not select more than 2 additional lesions for targeting.

As the study will be performed as a microdosing study, risks concerning the tracer mass are not to be expected. All subjects will be subject to small amounts of research-related ionizing radiation.

There is also a possibility of breach of confidentiality.

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Procedures for enrolment

Subjects will be recruited at Michigan Medicine.

7.2 Inclusion criteria

Male subjects (≥ 18 years of age) with prior mpMRI (within 3 months of recruitment) resulting in at least one PI-RADS 3-5 lesion with clinical need for a prostate biopsy procedure can participate, if one of the 2 criteria apply:

- Known Gleason 6 (3+3) or 7 (3+4, 4+3) prostate cancer undergoing active surveillance or in the confirmation phase of active surveillance,

  or

- Rising PSA with either a) no prior biopsy or b) single or multiple negative prior biopsies.

7.3 Exclusion criteria

Subjects cannot participate in this study if any of the following applies:

- Prostate biopsy within 12 weeks prior to enrollment
- Acute prostatitis within the last 6 months
- Current non-urologic bacterial infection requiring active treatment with antibiotics
- Active other malignancy (except basal cell or squamous cell skin cancer) within the last 2 years
- Body weight greater than 350 lbs (158 Kg)
- Inability or unwillingness to receive a prostate biopsy procedure
- Unable to lie flat, still, or tolerate a PET/CT scan
- Unable to provide own consent
- Concurrent severe and/or uncontrolled and/or unstable medical disease other than prostate cancer (e.g. poorly controlled diabetes, congestive heart failure, myocardial infarction within 6 months prior to study participation, unstable and uncontrolled hypertension, acute renal failure of any intensity, chronic renal or hepatic disease, severe pulmonary disease)
- Prisoner.

Subjects who received a ^{68}Ga-PSMA PET/CT and subsequent (clinical) TRUS-guided targeted biopsy, and are again in clinical need of a prostate biopsy can again take part in the study to receive repeat ^{68}Ga-PSMA PET/CT imaging.

7.4 Withdrawal and termination criteria

7.4.1 Subject withdrawal

There are no formal withdrawal criteria for this study. During the conduct of the study, the investigator will review for signals that would indicate the need for withdrawal of a subject.

In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time. Investigators also have the right to withdraw subjects from the study in the event of illness, AEs, or other reasons concerning the health or well-being of the subject, or in the case of lack of co-operation.

Should a subject withdraw after administration of the radiotracer, or should the investigators decide to withdraw the subject, all efforts will be made to complete and report the protocol-stipulated observations up to the time of withdrawal as thoroughly as possible. A final evaluation at the time of the subject's withdrawal should be made and an explanation given of why the subject is withdrawing or being withdrawn from the study. The reason and date and time of withdrawal must be noted in a case report form (CRF). If the reason for withdrawal is an AE, monitoring will continue until the outcome is established.

7.4.2 Study termination

The principal investigator reserves the right to terminate the study on safety grounds at any time. On termination of the study, the investigators will assure that adequate consideration is given to the protection of enrolled subjects' interests. Termination of the study will be considered in the event of significant adverse safety findings occurring at any time during the performance of the study. Should termination be necessary, the IRB, the local health authority, and any other relevant local or national health authority (FDA) will be notified.

8 TREATMENT OF SUBJECTS

In nuclear medicine imaging, it is important to record sufficient numbers of (true) positron annihilation events in VOIs in order to produce images of good quality, which can reliably be interpreted and deliver reproducible results. The number of recorded events increases with the duration of the acquisition period. Furthermore, for a given duration of image acquisition and for a given imaging time post injection, image quality increases with the administered radioactivity over a wide range of radioactivity doses. Therefore, the injected radioactivity should be high in order to obtain good images. On the other hand, higher radioactivity doses cause higher radiation exposure to the subjects, which is to be avoided. The optimum radioactivity dose is defined as the lowest dose that provides high image quality for a given duration of image acquisition and for a defined imaging time post injection.

8.1 ^{68}Ga-PSMA

8.1.1 Investigational medicinal product preparation

Gallium-68 labeled PSMA-11 (or PSMA-HBED-CC) is a radiopharmaceutical that will be produced under cGMP.

8.1.2 Treatment administration

The amount of ^{68}Ga-radioactivity administered for a single study will range between 3.0 and 7.0 mCi.

8.2 Method of numbering subjects

At screening, subjects will be given screening code numbers (starting from sc001, ect.). Once enrolled, subjects will be assigned unique subject numbers. Subject identification numbers will start from 001 to the maximum recruitment number.

8.3 Blinding

This will be an open-label study and no blinding will be performed.

8.4 Prior and concurrent therapy

Any prior, concurrent, or procedural medications or therapy given to or taken by the subject within 21 days before and up to the time of PET imaging will be recorded in the CRF along with the indication. Either the generic or the trade name may be recorded.

8.5 Treatment compliance

Subjects will receive radiotracers under direct supervision of qualified study personnel. Each administration volume and total amount of radioactivity injected will be checked, and the details—including date and time of injection—will be recorded.

9 TREATMENT PERIOD

Informed consent will be obtained. All subjects must satisfy one inclusion criterion and none of the exclusion criteria listed. Signed and dated informed consent must be obtained from all subjects before any study-specific procedures are performed. The consent document may be sent to a potential participant prior to obtaining consent for their information. A screening visit will be performed within 2 months before radiotracer administrations, however can be performed on the day of PET/CT imaging. Subjects will be permitted to continue taking any routine or necessary medication.

The following data will be collected on study-specific CRFs.

- Date of birth
- Weight
- Height
- Concurrent medications
- Medical history and concurrent diseases
- Report and date of most recent histological tissue evaluation from any known prostate cancer
- Results of screening tests

Personal data (including contact information) will be collected with the subject's permission and only to the extent that is necessary for the purposes of the study.

Vital signs (pulse, respirations, systolic and diastolic blood pressure) are recorded. A cannula (or indwelling catheter) will be placed into a vein, preferably in the antecubital region, for tracer administration.

The injection site will be examined for any abnormal findings before dosing. In the interests of comfort, subjects will be advised to empty their bladder and, if necessary, complete a bowel movement before being positioned on the scanner table.

9.1 ^{68}Ga-PSMA PET/CT

a) ^{68}Ga-PSMA-11 PET preparation and injection:

   The injected dose will be 111-259 MBq (3-7 mCi) of ^{68}Ga-PSMA-11 PET. PET imaging will begin 50-100 minutes after injection. Scan time per bed position will be determined based on expected count statistics related to the administered dose.

b) Patient preparation: no fasting is required.

c) PET protocol: Scan coverage will extend from mid-thigh to the base of the skull, starting from the mid-thighs to prevent urinary bladder radiotracer accumulation at the start of PET imaging. Scan time per bed position will be determined based on expected count statistics, which is determined mainly by the administered dose. Scan time will therefore be between 15 and 40 minutes. In certain circumstances, coverage may be extended to the toes. Contrast may be administered as needed.

d) Patient monitoring: Vital signs will be assessed immediately before and after injection of ^{68}Ga-PSMA-11 (HR and supine BP). Patients will be monitored for adverse events during injection and for two hours

after radiotracer administration. Additionally, patient's vitals (HR and supine BP) will be checked at the completion of the imaging study prior to leaving the imaging center.

e) Patient follow-up: Patients will be contacted by phone one to three days after ^{68}Ga-PSMA-11 PET to assess for the development of delayed adverse events. Patients will be seen in the clinic if there are any concerning study related adverse events requiring further evaluation.

9.2 Post-Treatment Period

We will adhere to the follow-up procedures specified in IND #133858. Patients are encouraged to self-report any possible side effect to the study team.

9.2.1 Target identification

In-vivo imaging (PET and/or MRI) will be reviewed by participating investigators for intraprostatic lesions suspicious for significant prostate cancer. After registration of imaging data to the T2-weighted MRI, the responsible radiologists (Dr. Davenport and Dr. Piert, or designees) will determine potential target areas for image-guided (targeted) prostate biopsy within the T2-weighted MRI (reference space). Candidate targets are the known PI-RADS 3-5 lesions, where higher PI-RADS scores will be preferentially selected. Any PI-RADS 5 lesion will be targeted for biopsy (unless there are more than 3). ^{68}Ga-PSMA positive lesions not identified by mpMRI as PI-RADS 3-5 will be considered as well. Currently available preliminary retrospective data suggest that a suitable threshold for ^{68}Ga-PSMA positivity (defining significant disease) would be ≥ 2 standard deviations above prostate background.[32]

To limit the burden to the subject, no more than 3 target locations will be selected for biopsy. If more than 3 targets are present (from both PET and/or MRI), the most suspicious 3 are selected; in case of a tie, the largest will be selected. The selected targets are then transferred to the navigated ultrasound workstation used for targeted prostate biopsies.

Radiomics analysis results will not be used to identify targets for prostate biopsies.

9.2.2 Prostate biopsy procedure

Prostate cancer subjects recruited for this study are expected to receive tissue sampling for clinical (diagnostic) purposes. Accordingly, all subjects already had a prior mpMRI indicating presence of a prostate lesion potentially representing prostate cancer (PI-RADS 3-5).

Targeted (fusion) prostate biopsies based on TRUS-MRI guidance are routine clinical practice at Michigan Medicine. While typically all biopsy targets are sampled, it is at the discretion of the physician performing the prostate biopsy which of the selected targets are biopsied. Typically, a total of 2 sample cores are obtained from each target. However, to account for biopsy cores missing the target area the exact number of biopsy cores obtained from a given target is at the discretion of the physician performing the prostate biopsy procedure.

The histological results of the biopsy will be compared to PI-RADS scores and respective visual and quantitative radiotracer uptake measures.

Patient monitoring after the prostate biopsy procedure will be done according to standard of care. The patient will receive specific instructions and guidance for minor and more serious adverse effects.

9.2.3 Pathological Evaluation

The histological confirmation serves as standard of truth. All biopsy cores obtained, whether targeted or standard, will be assessed for diagnosis.

Routine histology of all prostate biopsy cores will be performed according to the established institutional guidelines. Biopsy samples will be processed for routine histological assessment (hematoxylin/eosin (HE) stain) using the paraffin embedding process and 3 µm sections. Each tumor focus will be assigned a primary and secondary Gleason grade and staged according to recent American Joint Committee on Cancer (AJCC) guidelines. Some cuts may remain unstained for later immunohistochemistry (IHC) for PSMA and/or other additional PIN cocktail (cocktail of basal markers and AMACR) IHC, when deemed necessary for clinical purposes [54].

9.2.4 Clinical decision making

Once the results of the pathological evaluation of the prostate biopsies are available, - in consultation with the patient - the treating physician (or treatment team) will decide the further clinical management. If the decision for treatment is made and the patient decides to receive prostatectomy at our institution (Michigan Medicine), an effort will be made to analyze the location of tumor lesions within the resected prostate specimen and to register pre-biopsy PET and MRI imaging with pathology. If feasible, the prostate specimen may undergo additional (research) MRI to facilitate the registration process [55].

9.2.5 Image interpretation and correlation with standard of truth

PET and MR imaging will be assessed visually. Abnormalities found on MRI and ^{68}Ga-PSMA PET will be selected as targets for image-guided biopsies.

Once prostate biopsies are obtained, the location of each biopsy is retrospectively associated with a matching region on pre-biopsy imaging (facilitated via image co-registration) and semi-quantitative PET data are obtained and compared with the standard of truth (Gleason score histology). Accordingly, we will determine whether a selected target was true positive, false positive, true negative or false negative on imaging (MRI and ^{68}Ga-PSMA PET) relative to pathological proof. Please note that the location (in 3D space) of a given biopsy core is known from ALL biopsies, whether obtained as standard or targeted core. This is possible because the navigated ultrasound workstation (i.e. UroNav, Invivo) used will record the biopsy core location (trajectory and core length within the gland) in 3D for all cores obtained [55,56].

Accordingly, the sensitivity and specificity for the detection of low-grade (Gleason 3+3) and significant PCa (Gleason ≥ 3+4) is determined for both modalities, ^{68}Ga-PSMA PET (based on uptake measures) and mpMRI (based on PI-RADS scores).

9.3 Lesion localization variables

9.3.1 Overall lesion localization rate

The total number of focal lesions detected on ^{68}Ga-PSMA PET/CT images will be recorded and compared to mpMRI.

9.3.2 Cancer lesion localization rate

The number of cancer lesions, as verified in histopathology, detected will be recorded and compared with co-localized lesions on ^{68}Ga-PSMA PET and mpMRI.

9.3.3 Visual assessment of lesion contrast and scoring

The investigator will visually assess the ^{68}Ga-PSMA PET/CT images. Using a scoring system (no increased uptake = 0, mild uptake = 1, moderate uptake = 2, high uptake = 3, relative to background) the accumulation of ^{68}Ga-PSMA in each target volume will be assessed.

9.3.4 Extra-prostatic lesions

We will also determine frequency and location of ^{68}Ga-PSMA positive lesions. Such lesions could be extra-prostatic tumor extension, lympho-nodal and/or distant metastatic disease not identified by conventional (standard) imaging. As clinically indicated (clinical relevance, accessibility, risk), an attempt will be made to obtain pathological proof of such lesions. The decision for or against a biopsy is made by the treating physician in consultation with the patient, and not demanded by the study.

9.3.5 Semiquantitative assessment: SUV, SUVR

The standardized uptake value (SUV) is defined as follows:

SUV = activity (Bq/cm/[(body weight (g)/injected activity (Bq)]

An even distribution of the tracer in the body results in an SUV = 1.

SUVRs can be obtained by calculating the ratio of SUV in a lesion and the SUV of a corresponding reference tissue, such as non-cancerous/non-inflammatory tissue of the same organ, blood, or muscle.

9.4 Adverse event monitoring

The presence of any pre-administration event (baseline signs and symptoms present just before radiotracer administrations) will be recorded in the CRF.

The following information will also be recorded in the CRF:

- The date of evaluation
- The onset time
- The resolution time or duration
- Action taken
- Status of symptom
- Intensity

Of these events, only events that increase in intensity after radiotracer administrations will be recorded as AEs in the CRF.

9.4.1 Adverse events

For this study, an AE is defined as any untoward medical occurrence in a clinical investigation subject after administration of the radiopharmaceutical products. An AE does not necessarily have to have a causal relationship with administration of the radiopharmaceutical product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated

with the use of ^{68}Ga-PSMA, whether considered related to that product, or not. Only symptoms/signs that begin or worsen in severity and/or frequency after ^{68}Ga-PSMA administration/use will be recorded as AEs in the CRF.

The AE profile of ^{68}Ga-PSMA in humans has been established. No major adverse events have been reported in the literature. Therefore, major adverse events are extremely unlikely to occur. However, study personnel will remain vigilant at all times to the occurrence of AEs, particularly those that may be life-threatening. Personnel who are trained in the acute management of anaphylaxis and other emergencies and who have access to appropriate clinical supplies will be immediately available while the subject is confined to the study center. Treatment of serious AEs (SAEs) will consist primarily of the support of vital functions.

The subjects will be closely observed and questioned for any kind of AE during the study procedures and at follow-up appointments throughout the study period with non-leading questioning (e.g., “How do you feel?”). The subjects will be instructed to immediately report any symptoms and signs to the study staff (i.e., between formal observations).

9.4.2 Serious adverse events

An SAE is defined as any AE that:

- Results in death
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is another important medical event.*

(*Other important medical events are those that may not result in death, be life-threatening, or require hospitalization, but may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical intervention to prevent one of the outcomes listed above.)

9.4.3 Adverse event and serious adverse event reporting

All AEs should be recorded using acceptable diagnoses, if possible. If an AE has already been reported, it is not necessary to report each individual sign and symptom of that AE as a separate AE. Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
Grade 4 Life-threatening consequences; urgent intervention indicated.
Grade 5 Death related to AE.

SAEs will be recorded in the CRF if they occurred as follows:

- After a subject first received ^{68}Ga-PSMA and throughout the subject's follow-up period*, whether or not the AEs considered related to the tracer injections, and
- After the subject's follow-up period, if a causal relationship to ^{68}Ga-PSMA cannot be ruled out.

(*Follow-up period is defined as the protocol-stipulated period or, for subjects prematurely withdrawn from a study, the duration of a subject's participation.)

All serious and non-serious AEs must be followed for a final outcome until the end of the follow-up period. An outcome of "unknown" is not considered to be an acceptable final outcome. An outcome of "not yet resolved" is an acceptable final outcome for non-serious AEs at the end of a subject's participation in a study, and for SAEs at database lock.

The PI will report all SAEs to local health authorities, the IRB, and investigators as required by local regulations.

9.4.4 Expedited reporting

Expedited Reporting to the Food and Drug Administration

As the study is being conducted under an IND, the Sponsor-Investigator is responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32).

The Investigator must report in an IND safety report any suspected adverse reaction that is both serious and unexpected. The Sponsor-Investigator needs to ensure that the event meets all three definitions:

- Suspected adverse reaction
- Unexpected
- Serious

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after the Investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

9.5 Other variables

9.5.1 Demographic data

The subject's demographic data (gender, age, ethnicity and race, weight, height) will be recorded at screening.

9.5.2 Medical and surgical history

The subject's relevant medical and surgical history (i.e., a condition that is either active at enrolment or adversely impacts the subject's medical condition at enrolment) will be recorded at screening. Also, relevant

medical and surgical history may be viewed and recorded from digital patient records and optionally obtained from physicians involved in the care of the subjects.

9.5.3 Prior and concurrent medication

Information about medications taken by the subject during the study period will be recorded.

9.5.4 Appropriateness of measurements

All assessments and measurements are considered appropriate and regarded as standard medical practice.

10 DATA HANDLING AND QUALITY ASSURANCE

10.1 Clinical data management

All adverse events will be followed with appropriate medical management until resolved. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

This trial will be monitored in accordance with the NCI approved University of Michigan Rogel Cancer Center Data and Safety Monitoring Plan. The study team will meet every 3 months or more frequently depending on the activity of the protocol. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness. At these regular meetings, the protocol specific Data and Safety Monitoring Report form will be completed and signed by the Principal Investigator or by one of the designated co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Rogel Cancer Center Data and Safety Monitoring Committee every 3 months for independent review.

10.2 Archiving

All study documentation at the investigators' site will be archived for a minimum of 7 years following completion or discontinuation of the study.

11 STATISTICAL METHODS AND PLANNED ANALYSIS

11.1 General statistical considerations

Statistical analysis will be performed as specified in the statistical analysis plan.

All subjects who complete the study without major changes versus protocol will be included in the evaluation of pharmacodynamics / pharmacokinetics.

11.2 Subject characteristics

A table will be provided with the following information:

- Number of subjects enrolled
- Number of subjects included in the efficacy analysis
- Number of subjects withdrawn from the study and the reason for withdrawal.

Demographic information (age, height, weight) will be summarized using descriptive statistics.

Medical histories will be summarized by counts and percent. Concurrent medications will be recorded and coded using a standard classification system and grouped by primary and secondary classes, if applicable.

11.2.1 Demographic and other baseline characteristics

Summary statistics (arithmetic mean, standard deviation, median, minimum and maximum for quantitative variables) will be presented. Frequency tables for qualitative data will be provided. Medical history findings will be summarized.

11.2.2 Pharmacodynamical data

Quantitative data will be described by the following summary statistics: arithmetic mean, standard deviation, median, minimum and maximum. Frequency tables will be provided for qualitative data.

11.2.3 Development of decision rules

Since mpMRI has already been performed prior to ^{68}Ga-PSMA PET/CT, and since suspicious lesions have been found and rated according to PI-RADS, the addition of ^{68}Ga-PSMA can lead to a) a change or confirmation of the mpMRI-defined PI-RADS risk score of a given lesion, or b) identification of additional lesions not identified on mpMRI. Since mpMRI has a high sensitivity, but relative low specificity for significant PCa, we anticipate that modification of the mpMRI risk score will be the main source for a change in management. Currently, all PI-RADS 3, 4 or 5 lesions found at mpMRI are targeted for biopsy. Particularly problematic is that while PI-RADS 3 lesions are very frequent, their probability for significant PCa disease is low. The second most likely outcome, PI-RADS 4, has a roughly 50% chance to result in significant disease. If absence of ^{68}Ga-PSMA uptake in such lesions is associated with lack of significant disease, hybrid ^{68}Ga-PSMA PET/MRI could replace invasive biopsy procedures and avoid side effects for prostate biopsies in many men. On the other hand, if focally elevated ^{68}Ga-PSMA uptake would be found to correlate with significant disease, such potentially lethal disease could be found at earlier time points and with higher precision. Accordingly, the data are acquired to subsequently develop an evidence-based decision rule to inform which patients would a) benefit from a prostate biopsy procedure, and b) benefit most from ^{68}Ga-PSMA in addition to mpMRI.

12 STATISTICAL EVALUATION

Accrual potential: At the University of Michigan, over 800 patients receive a prostate biopsy per year (812 in 2016, 652 in 2015, 444 in 2014). The number of navigated TRUS-MRI fusion (targeted) prostate biopsies is rapidly increasing and reached 310 in 2016. In 2016, the Department of Radiology performed 960 mpMRI studies, of which about 60% (576) had a PI-RADS score ≥3.

Prevalence of significant PCa: Among all patients with PI-RADS score ≥3, the incidence of Gleason ≥ 3+4 PCa is 15% for PI-RADS 3, 55% PI-RADS 4, and 80% PI-RADS 5. At our center, the sensitivity/specificity for Gleason ≥ 3+4 is 96.5/59.7% for PI-RADS ≥3, and 78.9/78.9% for PI-RADS ≥4. The patient population for this trial will closely resemble a recently retrospectively investigated surveillance population from 2 centers in the state of Michigan (n=207) of men with clinical stage T1/T2 Gleason 3+3 PCa. The distribution of PI-RADS grading was at follows (unpublished data): PI-RADS 1-2 (37%), PI-RADS 3 (19%), PI-RADS 4 (31%), PI-RADS 5 (13%). This incidence of PI-RADS scores ≥ 3 is used in **Table 1** to estimate the incidence of Gleason 3+3 vs. ≥ 3+4 vs. benign findings in the trial population. Since we will only select patients with prior mpMRI resulting in PI-RADS 3-5, we will be able to select a specific relevant patient population with a sufficient risk for significant disease (defined as Gleason ≥ 3+4 PCa).

Disease incidence in study population: We estimate the potential impact of molecular imaging in addition to mpMRI for this trial on the basis of our prior ^{18}F-choline PET/MRI trial.[16] Our final (submitted) data included 90 PI-RADS 3-5 lesions in 56 subjects resulting in 30 “positive” (Gleason ≥ 3+4) and 60 “negative” (Gleason 3+3 or benign). Using a logistic regression model with two predictors—MRI suspicion score and a ^{18}F-choline lesion-to-background ratio of ≥ 1.58 (AUC = 0.95) —strongly favored the combined model over the mpMRI model alone (AUC = 0.83, $p < 0.001$). The selection of all PI-RADS ≥ 4 lesions for the prediction of Gleason ≥ 3+4 PCa resulted in an excessive number of false-positive (n = 27 [FP]) and false-negative (n=3 [FN]) cases. When selecting PI-RADS 5 lesions only, 3 FP and 11 FN cases would have resulted, which indicates missing too many significant cancers. The addition of ^{18}F-choline PET to mpMRI resulted in 5 FP and 5 FN cases.

Compared to the selection of all PI-RADS ≥ 4 lesions (high sensitivity, poor specificity), the combined ^{18}F-choline PET/MRI model resulted in an improvement of the specificity by +38% with only a modest reduction in sensitivity (-10%). Implementation of this rule would have reduced the number of targeted lesions for biopsy by roughly 50% (from 51 to 25), exemplifying the cost-saving potential of a PET/MRI strategy. Given that ^{68}Ga-PSMA and ^{18}F-choline have different molecular targets (^{68}Ga-PSMA has higher specificity for PCa than ^{18}F-choline), and that the ^{18}F-choline trial investigated a high-risk patient population, these ^{18}F-choline results are not directly transferable to the use of ^{68}Ga-PSMA PET/MRI such as is being investigated in this proposal. Furthermore, any future ^{68}Ga-PSMA PET/MRI rule would likely be constructed differently. For sample size estimates in this trial, we therefore project conservatively that the sensitivity of mpMRI would minimally increase (by 2%, mainly from correctly identifying significant cancers rated as PI-RADS 3), while the specificity would increase by only 15%. Given the known superiority of ^{68}Ga-PSMA over ^{18}F-choline, as well as the known increase of PSMA-expression with increasing aggressiveness (Gleason scoring), we have no reason to assume that the performance of ^{68}Ga-PSMA would be inferior to ^{18}F-choline for the identification of significant PCa. As a result, these sample size estimates are quite conservative.

In conclusion, we are confident that the necessary number of subjects can be recruited with sufficient prevalence of significant PCa in this trial. The inclusion criteria will ensure a sufficient potential for positive imaging findings with ^{68}Ga-PSMA PET/CT, and a very high probability of successful targeting with image-guided biopsy.[16]

Table 1. Estimated incidence of Gleason 3+3 vs. ≥ 3+4 vs. benign biopsy results in the trial population

Maximum PI-RADS risk score per subject	**Number of subjects (%)**	**No prostate cancer Benign %**	**Total maximum grade Gleason = 3+3 %**	**Total maximum grade Gleason ≥ 3+4 %**
3	30	16 (53%)	10 (33%)	5 (17%)
4	49	9 (27%)	13 (27%)	26 (53%)
5	21	1 (5%)	3 (14%)	17 (81%)
Total (N)	100	26	26	48

Primary objective: Mixed-effects logistic regression is used to evaluate the primary objective, the diagnostic performance of mpMRI and PET. The outcome is Gleason ≥ 3+4 or not, and predictors are PI-RADS v.2 score and ^{68}Ga-PSMA PET uptake. We employ a mixed effects model to account for within-subject correlation among one or more biopsied lesions. We will test the null hypothesis that the regression coefficient of PET is equal to zero versus an alternative hypothesis that it is not equal to zero, assessing whether ^{68}Ga-PSMA PET is an important factor to identify aggressive PCa. We also compare predictive accuracies of (1) PI-RADS ≥3 only and (2) PI-RADS ≥3 plus ^{68}Ga-PSMA PET models by examining the confidence intervals of a predictive performance measure: positive likelihood ratio (positive LR).

Secondary objectives: We are not expecting any adverse events related to the administration of the radiotracer. However, frequency tables will be generated to display the safety profile of ^{68}Ga-PSMA (secondary objective).

We will also record the frequency and location of extra-prostatic ^{68}Ga-PSMA positive lesions (such as extra-prostatic extension, lympho-nodal and/or distant metastatic disease) not identified by conventional (standard) imaging. Formal statistical evaluation is not planned as the frequency of such findings is expected to be low.

Exploratory objective: We will evaluate whether radiomics classifiers (obtained from mpMRI and ^{68}Ga-PSMA identified targets) could improve identification and risk stratification of significant PCa. Reference standard will be pathology defined as targeted plus standard prostate biopsy, and prostatectomy, when available. The performance of the different classifiers in detecting significant PCa will be evaluated by using the area under the receiver-operating characteristic (ROC) curve (AUC) using pathology as gold standard.[57]

12.1 Sample size determination

Sample size calculation was made for the primary objective only. We assess potential differences between the predictive performances of the two models (1) and (2), while maintaining ethical and economic considerations. We consider the positive likelihood ratio (positive LR) as a performance measure, which is defined by a ratio of sensitivity to 1-specificity. A higher positive LR means greater predictive information of positive test results irrespective of disease prevalence. Based on preliminary data obtained from mpMRI, we assume that the sensitivity and specificity of model (1) are both given by 0.789. For model (2), we assume conservatively that the sensitivity will be increased by 0.02 (2%) and that the specificity would improve by 0.15 (15%). In this scenario, the positive LR of model (1) is given by 3.74 and the positive LR of model (2) is calculated as 5.02. In order to detect the 1.28 difference in positive LR between the two models, we need at least 100 subjects, including at least 38 of 100 subjects with a histological diagnosis of significant PCa. The sample size was estimated as described in the literature. [58]

12.2 Significance Level

All statistical tests will use a 0.05 significance level.

12.3 Rules for excluding subjects from analysis

All dosed subjects will be included in the analyses unless otherwise specified. The PI will make any decisions regarding whether any subjects or any individual values belonging to a subject who violates the protocol will be excluded from the evaluations when the protocol violation is considered to have a negative impact on the scientific aspects and interpretation of the study results. The PI will also make a decision whether recruited

subjects with incomplete assessments will be substituted with additional subjects beyond the total recruitment goal.

12.4 Withdrawal from assessment and replacement

Subjects who show major protocol deviations, e.g. deviations leading to the loss of essential data for the evaluation of the diagnostic efficacy can be withdrawn and replaced. The decision regarding withdrawal and replacement will be made by the principal investigator consulting with the study team.

12.5 Analysis Plan

12.5.1 Analysis Population

Patients with histopathology correlates will be analyzed for the primary aim. All remaining patients will be analyzed for the secondary endpoints.

12.5.2 Analysis of Primary Endpoints

PET images will initially be interpreted by a board certified nuclear medicine physician or a board-certified radiologist experienced in reading PET at the time of the imaging study at Michigan Medicine. Upon request, case report forms and coded ^{68}Ga-PSMA PET/CT imaging data will be provided to the FDA for review.

Intraprostatic lesions identified by mpMRI as PI-RADS 3-5 will be graded visually on a two-point scale as either positive or negative for elevated ^{68}Ga-PSMA uptake relative to prostatic gland uptake (0=Negative or 1= Positive).

Any focal intraprostatic elevated ^{68}Ga-PSMA uptake relative to prostatic gland that was not identified by mpMRI (as PI-RADS 3-5) but was targeted at biopsy will also be evaluated visually as either positive or negative (0=Negative or 1= Positive).

All subjects with histopathology correlates will be analyzed for the primary aim. Sensitivity and specificity, positive and negative predictive values, and accuracy (along with the corresponding two-sided 95% confidence intervals) will be determined on a per-subject and per-lesion basis for ^{68}Ga-PSMA-11 PET and mpMRI regarding the detection of Gleason ≥ 3+4 cancer. Standard reference of truth is histopathology from all individual biopsy cores, targeted and standard cores.

12.5.3 Analysis of Secondary Endpoints

All subjects receiving ^{68}Ga-PSMA will be included in the safety analysis.

Any extraprostatic lesion will be judged as positive if the lesion is visually positive with uptake greater of equal to 2 standard deviations above local background.

12.5.4 Analysis of the Exploratory Objective

Radiomics analyses will be performed retrospectively, thus after prostate biopsies have been performed. Accordingly, radiomics analysis results will not be used to identify targets for prostate biopsies.

Radiomics analysis consists of two steps: (1) Image feature extraction and (2) Model building of discriminant classifiers.[50] We plan to extract intensity, texture and morphological features from ^{68}Ga-PSMA PET and mpMRI[46] into mathematical models using advanced machine-learning methods while accounting for confounding effects from clinical parameters (stage, age, histology, etc.).[59] For feature extraction we will assess co-registered T1w, T2w, ADC, DCE MRI, and ^{68}Ga-PSMA PET volumes. Specific image features related to tumor heterogeneity include: (a) first-order histogram intensity variations (mean, min, max, variance, skewness, etc.); (b) shape (eccentricity, solidity, etc.), and (c) texture features (second order histogram metrics), which relate intensity to local spatial distribution using matrix methods such as the gray-level co-occurrence matrices (GLCM) and others such as neighborhood gray-tone difference matrix (NGTDM), run-length matrix (RLM), and grey-level size-zone matrix (GLSZM) features on PET,[46] PET/CT,[60] and separate or fused PET/MRI.[61] Image pre-processing of VOIs involves feature enhancement (denoising/deblurring) and rescaling to isotropic voxels.[46,50]

We will explore advanced statistical learning methods to build maximally discriminant classifiers of significant PCa (Gleason ≥ 3+4). Different machine learning classifiers using linear generalized models (Elastic net), support vector machine (SVM), random forests (RF), and deep learning (convolutional neural network (CNN)) techniques may be enployed.[62,63]

Methods based on statistical resampling and information theory will be used to guard against over-fitting and to evaluate generalizability to out-of-sample data.[64] Feature-based classifiers (LASSO, SVM, RF, NN, DNN) and featureless classifiers (CNN) may be applied as well.

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