A Phase II Study of ^{68}Ga-RM2 for PET/CT of Gastrin Releasing Peptide Receptor (GRPr) Expression in Prostate Cancer

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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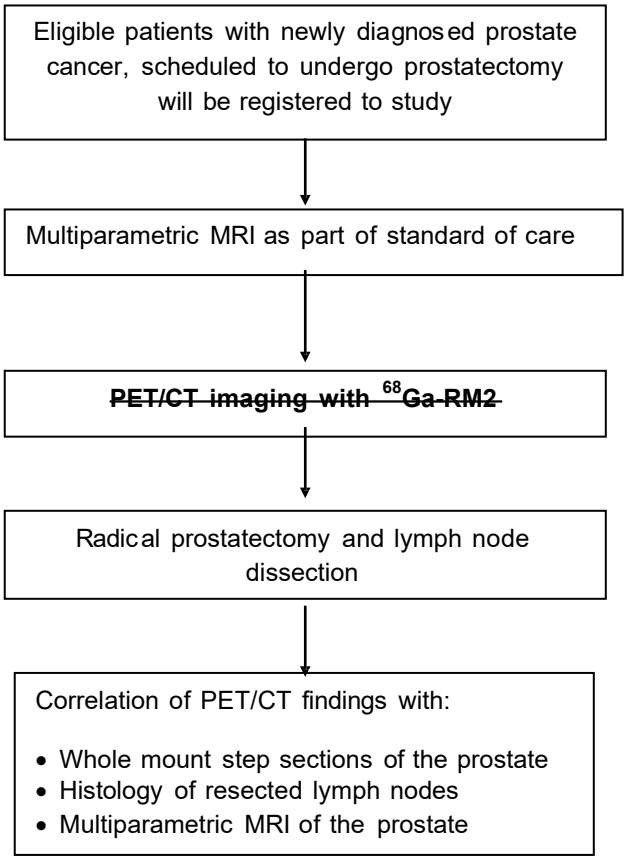
1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Schema



2.0 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Primary Objective

- To determine the sensitivity and specificity of ^{68}Ga-RM2 PET/CT to detect foci of primary prostate cancer using whole mount step sections of the prostate as a reference standard.

(^{68}Ga-RM2 is a Galium-68 labeled, gastrin releasing peptide receptor RM2 [DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH_2]).

2.2 Secondary Objectives

- To compare the findings on ^{68}Ga-RM2 PET/CT in the prostate with multiparametric MRI.
- To study biological correlates for the increased GRPr (gastrin releasing peptide receptor) expression of prostate cancer.

2.3. Exploratory Analysis

- To obtain preliminary data on the ability of ^{68}Ga-RM2 PET/CT to detect lymph node metastases. Because of the limited number of high risk patients included (n=6) we expect only few lymph node metastases. The ability of 68Ga-RM2 PET to detect these metastases will be reported in a descriptive fashion.
- To obtain further descriptive data on the safety of ^{68}Ga-RM2.

3.0 BACKGROUND AND RATIONALE

3.1 The Problem Being Addressed:

In 2013, an estimated 238,540 US men will receive a diagnosis of prostate cancer and 29,170 are expected to succumb to the disease (1). If we compare this to 1988, the incidence today is 2.4 times higher but the mortality estimate is the same. The Swedish randomized trial of radical prostatectomy versus watchful waiting demonstrated a 50% reduction in metastasis and cancer specific mortality (2). In our experience of about 1700 patients treated with surgery alone, 15-year estimates for PSA progression and death from disease were 26% and 7% respectively. Despite these excellent rate of cancer control, perioperative morbidity (e.g., acute surgical and anesthetic complications, need for transfusions, incision pain, and time away from normal activities) is still too common. While some of these events have improved with minimally invasive surgery, other key outcomes measures such as positive surgical margins, urinary incontinence and erectile dysfunction remain unacceptably high. A patient with clinically localized cancer who is unlikely to harbor an indolent tumor and is contemplating surgery must have a clear understanding of the treatment goal: complete removal of the prostate, seminal vesicles and nodes (if indicated) with a negative margin and complete preservation of continence and potency. This is no easy task. The operation is technically challenging and its outcomes are highly sensitive to fine nuances in the surgical technique. Knowledge of cancer location, aggressiveness, size and extent are essential if treatment is to be tailored to individual patients.

The need for individualized therapy of prostate cancer has been underlined by a large multicenter study on the mortality of prostate cancer (3). In this study the 15-year prostate cancer specific mortality rate was estimated at 7%, with poorly differentiated cancer and seminal vesicle invasion. In contrast, patients with Gleason score 6 or less had the lowest risk of prostate cancer specific mortality (0.03%). This data clearly demonstrates the wide range risk spectrum of prostate cancer and forms a strong impetus for a risk-adjusted management for prostate cancer.

Advances in imaging have potential for achieving the goal of individualized treatment. As most cancers treated today are not palpable, the information from imaging will help the surgeon improve preoperative staging and deliver a more efficient surgical strategy. Imaging is also necessary for focal therapies of prostate cancer, such as radiotherapy or focused ultrasound (4).

Management of patients with these low-risk tumors could be significantly improved by imaging techniques that accurately visualize the tumor in the prostate and allow for serial non-invasive imaging studies. Current active surveillance strategies require repeated biopsies in addition to digital rectal examination and PSA testing (5, 6).

Thus there are four unmet medical needs that could be addressed by improved imaging technologies: (i) better identification of clinically significant prostate cancers, (ii) better delineation for prostate cancer for focal therapies, (iii) accurate non-invasive detection of lymph node metastases and (iv) non-invasive ways to monitor patients during active surveillance. The goal of this trial is to obtain initial clinical data whether PET/CT with a new ligand, targeting the gastrin releasing peptide receptor (GRPr) can address these needs.

3.2. Existing Imaging Modalities in Prostate Cancer

The cross-sectional imaging modalities that are used to evaluate prostate cancer include transrectal ultrasound (TRUS), x-ray computed tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS) as well as positron emission tomography with fluorodeoxyglucose (FDG) or choline.

3.2.1 Computed tomography (CT)

CT generally cannot detect disease that is confined to the prostate because of the limited contrast between tumor and normal prostate. In patients with high risk disease CT is used to exclude macroscopic lymphadenopathy. However, the sensitivity of CT to detect lymph node metastases is limited, because metastases frequently do not cause a significant enlargement of the node at the time of diagnosis. A meta-analysis of 24 studies has concluded that the pooled sensitivity of CT for detection of lymph node metastases was 42% at a specificity of 82% (7).

3.2.2 Transrectal Ultrasound (TRUS)

Transrectal ultrasound was introduced in the 1970s and is used to localize areas suspicious for prostate cancer and directing transrectal biopsies (8). TRUS visualizes the seminal vesicles and the ampulla of the vas deferens. TRUS imaging also allows to differentiate the peripheral hyperechogenic zone from the central hypoechogenic zone. These anatomical features are used to guide systematic transrectal biopsies. Prostate cancer is typically visualized as hypoechogenic area within the prostate. However, up to 50% of prostate cancer show a similar echogenicity than the peripheral zone which limits the sensitivity of the technique for prostate cancer detection. Biopsies of prostate cancer guided by TRUS have shown only a poor correlation with the highest Gleason score as determined from systematic biopsies or prostatectomy specimens. In a multicenter study including almost 4000 patients, prostate cancer was detected in 25% of the hypoechogenic and in 25% of the isoechogenic lesions (8).

3.2.3 Magnetic resonance imaging and spectroscopy (MRI/MRS)

Magnetic resonance imaging has been extensively studied for the detection and staging of prostate cancer as well as for differentiating clinically significant prostate cancers from insignificant cancers (9). Commonly used MRI techniques include T2 weighted and diffusion weighted images as well as contrast enhanced, T1 weighted images. The sensitivity and specificity for detection of prostate cancer varies with the used techniques and higher diagnostic accuracy is obtained by combining different imaging features (multiparametric MRI). MRI provides excellent soft-tissue resolution and is the most sensitive anatomic imaging modality available for the detection and staging of localized prostate cancer. A standard prostate examination includes at least multi-planar T1 - and T2 -weighted imaging and diffusion-weighted MRI (DW-MRI). These sequences are frequently complemented by T1 weighted contrast

enhanced study (dynamic contrast enhanced MRI, DCE-MRI). On T2 weighted images, prostate cancer usually demonstrates decreased signal intensity within the high-signal-intensity peripheral zone. However, the detection of prostate cancer can be hampered by the presence of confounding factors, such as post-biopsy hemorrhage and chronic prostatitis (9).

DW-MRI allows for the measurement of water diffusivity. The degree of water diffusivitiy can be quantified by the apparent diffusion coefficient (ADC), a measure of average molecular motion in the intra- and extracellular space (10). Generally, prostate cancer is detected as an area of low ADC. DCE-MRI involves the assessment of changes in signal intensity over time after contrast injection. During DCE-MRI, the contrast agent gadopentetate dimeglumine (Gd-DTPA) is injected intravenously and passes from the intravascular space to the interstitial space at a rate that depends on perfusion, vascularity, extracellular volume and vascular permeability. Prostate cancer typically demonstrates rapid initial uptake and rapid clearance of the contrast agent. The signal from DCE MRI can quantified by various parameters including K^{trans} a parameter describing the transfer of contrast from the vascular space to the extracellular space and kep which describes the transport from the extracellular space to the vascular space (9).

Magnetic resonance spectroscopy (MRS) provides maps of the distribution of choline, creatine, citrate and polyamines. Prostate cancer typically shows an increased concentration of choline and creatine relative to citrate (9).

While multiparametric, multiplanar MRI is currently the most accurate technique to detect prostate cancer, sensitive and specific detection of prostate cancer remains challenging, especially for tumors with low Gleason scores. Furthermore, MRI signals do not accurately reflect Gleason scores.

Detection of lymph node metastases on MRI is based on the size of the lymph node and therefore faces similar limitations than CT. A meta-analysis reported a pooled sensitivity and specificity of 39% and 82% (7). MRI is highly accurate for the diagnosis of bone metastases of prostate cancer and is frequently used 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.

3.2.4 Radionuclide bone scanning

In prostate cancer, bone scintigraphy is highly sensitive for detection of bone metastases, because prostate cancer metastases typically induce strong osteoblastic response. However, radionuclide bone scanning does not image tumor, but the reaction of the normal bone matrix to the metastasis. Thus it provides only a direct measure of cancer activity. Furthermore, the bone tissue reacts in a similar way to trauma, infection and non-physiologic stress. Consequently the specificity of radionuclide bone scanning is low. In addition, the common occurrence of the flare phenomenon (a paradoxical increase in intensity after successful therapy due to healing of the bone matrix) complicates the interpretation of scans on a serial basis.

3.2.5 Positron Emission Tomography (PET)

PET has advantages over other imaging methods because it quantitatively assesses biologic processes in vivo and can assess different processes using specific radiotracers. Processes that can be analyzed with currently available reagents include glucose and amino acid metabolism

and proliferation, blood flow, and receptor status (i.e., androgen receptor). However, most studies have focused on the accumulation radiolabeled choline or choline analogs and FDG.

3.2.5.1 FDG PET

During the past decade, investigators at MSKCC have shown that PET imaging in prostate cancer can be tested prospectively using rigorous, carefully controlled standard clinical trial designs traditionally reserved for therapeutics. FDG PET has been shown to be clinically valuable to differentiate between active and inactive/treated metastases in castration resistant prostate cancer (11, 12). FDG PET was also found useful for monitoring chemotherapy in patients with metastatic prostate cancer. However, FDG uptake of primary prostate cancer is typically low, and the sensitivity of FDG PET for detection of primary prostate cancer is therefore limited.

3.2.5.2 *Choline PET*

^{11}C-Choline has recently been FDA approved for detection of recurrent prostate cancer. ^{11}C-Choline as well ^{18}F labeled choline analogs (fluoroethylcholine and fluoromethylcholine) are commonly used in Europe for restaging of prostate cancer in patients with rising prostate specific antigen (PSA) levels following radical prostatectomy or radiotherapy for prostate cancer(13, 14). Choline PET has also been studied for detection of primary prostate cancer and of lymph node metastases at the time of initial diagnosis. While initial studies reported a high sensitivity and specificity for detection of prostate cancer, more recent studies have indicated that choline is also significantly accumulated by benign prostate hyperplasia and in chronic prostatitis (15). Systematic studies correlating choline uptake in the prostate with step-section whole mount histology found only a low sensitivity and specificity for detection of prostate cancer. Focal choline uptake in regional lymph nodes is quite specific for metastatic disease (specificity 94%), but the sensitivity was only 56% in a recent study including more than 200 patients with newly diagnosed prostate cancer (16).

In summary, a variety of clinically available imaging modalities have been studied for imaging of prostate cancer. Currently MRI is the most accurate imaging technology for detection and regional staging of prostate cancer. However, even MRI demonstrated significant limitations in the detection and delineation of prostate cancer as well as in the accurate detection of regional lymph node metastases. Consequently, there is a clear clinical need for novel approaches for prostate cancer imaging.

3.3 Gastrin Releasing Peptide Receptor (GRPr) in Prostate Cancer

An attractive target for a more sensitive and specific imaging of prostate cancer is the gastrin releasing peptide receptor (GRPr), a G protein coupled receptor that is a member of the bombesin receptor family. The bombesin family receptor is named after 14 amino acid peptide bombesin which was initially detected in the skin of the toad bombina. The bombesin receptor family comprises of four receptors, the BB1 or neuromedin receptor, the BB2 or GRPr receptor, the BB3 receptor which is not found in humans and the BB4 receptor for which the natural ligand is currently unknown (17).

GRPr and its human ligand, gastrin releasing peptide (GRP), are physiologically expressed in the central nervous system and the gastrointestinal tract (17). GRPr overexpression has been observed in a variety of malignant tumors including breast cancer, small cell lung cancer and (17), but most

consistently in prostate cancer. An autoradiographic study found markedly increased binding of radiolabeled bombesin by prostatic intraepithelial neoplasia (PIN) and all 38 primary prostate cancers studied. In contrast, normal and hyperplastic prostate tissue demonstrate no or very low binding of bombesin (18).

The potential of radiolabeled peptides in nuclear medicine to target tumor receptors has been investigated for many years and has been proven to be successful especially in the case of somatostatin. The radiotracer [68 Ga]DOTA-D-Phe1-Tyr3-Octreotid (DOTATOC) is used for the diagnosis of neuroendocrine tumors in clinical routine (19). Peptides provide excellent characteristics for PET-imaging because they can easily be synthesized, have fast and specific targeting features and are rapidly cleared from the body mainly via the renal pathway. In particular, peptides targeting G-protein coupled receptors are effective in accumulating in tumors in vivo. Peptides can also easily be labeled with therapeutic radionuclides for radiopeptide therapy of metastatic tumors. This ―theranostic‖ concept has been highly successful for imaging and treatment of neuoroendocrine tumors with the combination of ^{68}Ga-DOTATOC PET imaging ^{90}Y-DOTA-TOC therapy (19).

The physiologic ligand of GRPr in humans is gastrin releasing peptide (GRP), a 27 amino acid peptide. Both bombesin and GRPr are highly unstable in human plasma and therefore cannot be used as imaging or therapeutic agents. Several groups have therefore developed more metabolically stable bombesin analogs. Most of the compounds are GRPr *agonists* that have been tested in preclinical models of prostate cancer as well as in preliminary clinical trials (20). However, most of these ligands demonstrated high gastrointestinal uptake and insufficient metabolic stability in-vivo. Furthermore, GRPr agonists can cause acute side effects (abdominal cramps, vomiting) when administered at higher doses, as needed for radionuclide therapy (21). These limitations can potentially be overcome by radiolabeled GRPr *antagonists*. Non-radioactive labeled GRPr antagonists have been developed as anti-cancer agents, since GRP is involved in mitogenic auto- and paracrine signaling loops (17). These compounds were found to cause only minimal side effects in clinical trials (22).

Our group has developed several radiolabeled GRPr antagonists for imaging and radionuclide therapy of prostate cancer. In mice these tracers showed higher tumor uptake than GRPr agonists. In addition, the antagonists showed rapid clearance from tissues with physiologic expression (gastrointestinal tract and pancreas) while they were retained for more than 24 h in GRPr expressing prostate tumor xenografts (23, 24).

Of the studied GRPr antagonists, ^{64}Cu-AR-CBC-TE2A demonstrated the most favorable characteristics with respect to tumor uptake and tumor-to-organ-ratios (23). Because of the ^{64}Cu label ($T_{1/2}$=12.7 h) pharmacokinetics of ^{64}Cu-CBC-TE2A can be studied over several hours. Based on these encouraging characteristics we selected this compound for initial clinical studies.

In a pilot ^{64}Cu-AR-CBC-TE2A biodistribution and tumor uptake were in 4 patients with newly diagnosed prostate cancer at the University of Freiburg. Patient characteristics are summarized in table 1.

Nr.	Age	Result core needle biopsy: Gleason score, TNM classification, tumor site and extension	PSA (ng/ml)	Therapy
1	64	4/6 biopsies positive in left lobe (Gleason 6, T2), right lobe negative, tumor fraction 2%	10,0	^{125}I seed implantation
2	77	3/4 biopsies positive in right lobe (Gleason 7, T2a), left lobe negative, tumor fraction up to 40%	4,6	percutaneous radiotherapy and androgen deprivation therapy
3	70	Biopsies positive in right lobe (Gleason 7, T2b), left lobe negative, tumor fraction 10%	9,9	percutaneous radiotherapy and interstitial after-loading
4	73	Biopsies positive in both sides of the prostatic gland (Gleason 7b, T2b), tumor fraction >90% in left lobe, <1% in right lobe	26,2	prostatectomy and lymphadenectomy

Table 1. Characteristics of patients studied with ^{64}Cu-AR-CBC-TE2A

All 4 tumors were visualized on the PET/CT images. Importantly, good contrast between the tumor and normal prostate tissue was achieved with 20 min to 4 hours post injection. These data therefore indicate that a long lived radionuclide is not necessary for PET/CT imaging of GRPr expressing tumors.

For radiolabeling of peptides the positron emitter Gallium-68 (^{68}Ga) is particularly attractive. ^{68}Ga can be produced in radionuclide generator from the long lived parent radionuclide Germanium-68. This a significant advantage when compared to ^{64}Cu which is produced in a cyclotron with an expensive solid target that is difficult to handle. ^{64}Cu is therefore only produced at a few centers within the US. The physical half-live of ^{68}Ga is only 68 minutes which results in a low radiation exposure of the patients as compared to 64Cu (physical half-life 12 h). ^{68}Ga labeling of peptides is performed with the commonly used Chelator DOTA (diethylentetraaminopentaacetat). ^{68}Ga labeled peptides are in broad clinical use for imaging of neuroendocrine tumors in Europe.

Because of these favorable characteristics we are evaluating a ^{68}Ga labeled GRPr binding peptide ^{68}Ga-RM2 for imaging of prostate cancer.

3.4 Properties of ^{68}Ga-RM2 (BAY 86-7548)

3.4.1 Receptor affinity in-vitro

In vitro, RM2 demonstrated high and selective binding affinity to the GRPr (IC 50 7.7-9.3nM, K d 2.5-3.1nM) whereas no binding was detected to other bombesin receptor subtypes such as neuromedin B (25). RM2 showed antagonistic properties due to inhibition of GRPr receptor internalization in GRPr-transfected HEK-cells and Ca^{2+} mobilization in PC-3 cells. No species differences in GRPr binding affinity between mouse, rat and human receptors was found (25).

3.4.2 Tumor uptake and biodistribution in mice

High and specific tumor uptake of RM2 was found in mice bearing GRPr expressing human prostate cancer models: the androgen-independent PC-3 xenograft (14% ID/g at 1h p.i.) and the androgen-dependent LNCaP xenograft (6% ID/g at 1h p.i.). Low uptake in non-target organs resulted in high tumor-to-tissue (T/T) ratios at 1h p.i. favorable for PET imaging (e.g. T/Blood = 25, T/Muscle = 130 for PC3 tumors). RM2 was mainly excreted via the urinary pathway (~70% ID after 1h, >80% after 2h). (25)

3.4.3 Pharmacokinetics and Metabolism in mice

In vitro, it was shown that the compound has a high stability in serum and liver microsomes. Negligible degradation of [^{177} Lu]-labeled RM2 was detected in vitro when incubated in human serum over a time period of 96 h as analyzed by radio-HPLC. After 96h incubation 70% of the peptide was still intact. Microsomal stability of radioactive RM2 tested in human and mouse liver microsomes showed minor but cofactor-independent degradation of the tracer after 60 min incubation as analyzed by radio-HPLC (BAY 86-7548 investigators brochure). Metabolic profiling of BAY 86-7548 showed that 2 metabolites in minor amounts are generated in the presence of liver microsomes and hepatocytes of both rat and human. After intravenous administration of the non-radioactive precursor of ^{68}Ga-RM2 in rat the compound distributes rapidly into the tissues with an alpha half-life of t 1/2 of 0.07 h and is also rapidly eliminated with a beta half-life of 0.19 h. After intravenous administration of [^{68}Ga] labeled RM2 to mice it was shown that the radioactivity is mainly excreted renally via urine. In vivo evaluation of metabolism of the radioactive RM2 was performed in mouse using radio HPLC. In this study minor amounts of polar metabolites were found in plasma. In urine, one of these polar metabolites was also identified as dominant degradation product which increased over time, while intact peptide was still detected in the urine after 10-15 min.

3.4.4 Safety pharmacology studies

The pharmacological safety of ^{68}Ga-RM2 bombesin analog was subject to studies addressing effects on the central nervous system (integrated into extended single dose toxicity study), cardiovascular system and respiratory system. Since the studies were intended to address the effects of the chemical entity only, a mixture (500:1) of RM2 and stable Ga-RM2) which are the precursor and the non-radioactive analogue of ^{68}Ga-RM2 was dissolved in 0.9% NaCl and given to rats as a single intravenous injection. Doses were 0 (vehicle), 0.11, 1.05 and 10.5 mg/kg. The overall results of the in vivo safety pharmacology showed no adverse effects on central nervous system function and pulmonary function in rats. A transient slight increase in systolic and diastolic blood pressure was found immediately after administration of the high dose. The no observed effect level (NOEL) was 1.05 mg/kg suggesting a safety factor of 300 to the planned maximal human mass dose of 30 µg of the mixture of RM2 and 68Ga-RM2 per patient (conversion based on body surface area).

3.4.5 Safety, pharmacokinetics metabolism in humans

The safety, pharmacokinetics and metabolism of ^{68}Ga-RM2 were studied in 5 male volunteers (26). The ^{68}Ga radioactivity was rapidly excreted through the kidneys to the urinary bladder and accumulated predominantly in the pancreas and liver. Maximum peak

uptake of the total injected radioactivity was seen in the urinary bladder contents and the liver, with approximately 36% and 14%, respectively. The proportion of intact RM2 on total radioactivity in venous plasma declined over time, and 3 metabolites were detected by radio-HPLC. The proportions of unchanged ^{68}Ga-RM2 were 92%±9%, 68%±9%, 47%±6%, 29%±6%, 19%±2%, 16%±4%, and 15%±2% at 1, 10, 20, 40, 65, 100, and 150 min after injection, respectively. The in vitro investigations applying human hepatocytes revealed the metabolic pathways. Metabolite M-1 (68 Ga-DOTA chelate) appeared to be major, and M-2 (cleavage of amide bond at alanine–valine) and M-3 (hydrolysis of glutamine residue) were minor constituents of total radioactivity.

The unchanged tracer reached maximum plasma concentration after about 10 min and was eliminated from the circulation, with a terminal half-life of about 80 min. The total clearance was estimated to be about 55 L/h. No significant changes in vital signs, EKG and routine laboratory parameters were observed (26).

3.4.6 Uptake in human prostate cancer

A recent study of ^{68}Ga-RM2 in 11 patients with newly diagnostic prostate cancer reported a sensitivity of 88% and a specificity of 81% for detection of prostate cancer foci in 132 prostate regions (27). This study did not include detailed comparisons with multiparametric MRI and no detailed correlative studies, such as correlation with ETS transcription factor rearrangements, was performed. ^{68}Ga-RM2 also identified tumor recurrence in two of three patients with biochemical recurrence (27).

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

Patients with newly diagnosed prostate cancer scheduled to undergo radical prostatectomy are eligible to register for this study, providing all other eligibility criteria are met. Patients can be co-registered to therapeutic clinical trials, but may not receive any treatment between the PET study and the prostatectomy. 6 patients with low-risk, 8 patients with intermediate-risk and 6 patients with high-risk tumors according to NCCN guidelines (2.2014) will be included. Patients in ―very low‖, ―locally advanced‖, ―very high‖, and ―metastatic‖ risk categories of the NCCN guidelines will not be included in this study. We expect the information of RM-2 PET/CT to be most clinically relevant in the ―low‖, ―intermediate‖, and ―high risk‖ patients.

The classification of patients as low, intermediate and high risk is based on Gleason score, clinical T stage and serum PSA level. All of these parameters are routinely known before the planned surgical procedure and documented in the patient‘s medical record. We will create a spreadsheet with the number of patients in each risk group. As soon as the planned number of patients in a specific risk group has been accrued we will no longer screen for patients in this risk group.

The goal of this proposal is to systematically evaluate a ^{68}Ga labeled GRPr antagonist for imaging of primary prostate cancer using whole mount step section pathology as the reference standard.

The primary objective (specific aim 1) is to determine the sensitivity and specificity of PET/CT with the GRPr antagonist ^{68}Ga-RM2 to detect foci of primary prostate cancer. Approach: we will include 20 patients with newly diagnosed prostate cancer (Gleason score 6 to 8) scheduled to undergo radical prostatectomy. Localization will be defined using a total of 12 regions within the prostate. To facilitate correlation of radiotracer uptake with histology and MRI findings, PET/CT images will be coregistered with a T2 weighted MR images of the prostate (see specific aim 2). Radiotracer uptake within each region (expressed as standardized uptake value, SUV) will be assessed without knowledge of other imaging or clinical patient characteristics.

Secondary objective 1 (specific aim 2) is to compare GRPr PET/CT for localizing prostate cancer with MRI and to determine if the combination of ^{68}Ga-PET and MRI is more accurate for detection of prostate cancer than either ^{68}Ga-PET and MRI alone. Approach: MR imaging (established as standard of care at our institution) will include T2-weighted as well as diffusion weighted imaging. MR images will be read (i) blinded to the results of GRPr PET/CT and (ii) using fusion images of MR and PET. The level of suspicion for prostate cancer for both approaches will be recorded on a 5 point scale. We will compare the ROC curves, sensitivity and specificity of GRPr PET/CT, MRI and GRPr PET/MR using a McNemar's test adjusted for multiple regions.

Secondary objective 2 (specific aim 3) is to perform exploratory analyses of histological, immunohistochemical, genetic and gene expression features of the resected tumors in order to identify factors influencing ^{68}Ga-RM2 uptake. Approach: we will correlate ^{68}Ga-RM2 uptake with Gleason score, GRPr expression (as determined by immunohistochemistry), as well as the presence of ERG and ETV1 rearrangements. These rearrangements have recently been shown to be associated with increased GRPr RNA expression in patients with prostate cancer, but their relationship to GRPr protein expression and GRPr ligand binding is currently unknown. In order to undertand the relationship between AR signaling and uptake of ^{68}Ga-RM2 we also determine the correlation between AR staining on immunohistochemistry and an AR gene expression signature.

In an **exploratory analysis** we will record the frequency of lymph node metastases on histopathology and the diagnostic accuracy of ^{68}Ga-RM2-PET/CT to detect these metastases. Analysis will be performed on the basis of lymph node regions that can be identified on the PET/CT images to ensure that findings on PET are correctly matched with positive nodes on histopathology. Lymph node regions will include: internal iliac, obturator, presacral, external iliac, common iliac (each on the left and right side). For each of these anatomical lymph node regions an assessment will be made on the PET/CT images if there is focal ^{68}Ga-RM2 uptake (above regional background) on PET in a lymph node identified on CT.

4.3 Intervention

The intervention is the administration of a single dose of 150-200 MBq ^{68}Ga-RM2 (mass <= 30 µg) for imaging purposes. This will be followed by a 30-40 min PET/CT study after a waiting period of 60 min (+/- 10 min). Prior clinical experience suggests that imaging can be performed within 1 hour post injection (27, 28). MR imaging and prostatectomy will be performed as standard of care at MSKCC.

If less than 150 MBq of ^{68}Ga-RM2 are synthesized due to technical difficulties, patient can be injected with that amount. However, only a limited PET/CT will be performed over the region of interest, over the pelvis with acquisition time per bed position adjusted to the lower amount of injected radioactivity.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

The investigational product in this study is ^{68}Ga-RM2 (RM2), a [^{68}Ga] labeled peptide antagonist of the bombesin GRPr receptor, for positron emission tomography (PET) imaging of tumors. ^{68}Ga-RM2 will be synthesized, formulated and tested for release as described in section 7 of this IND application. The synthetic precursor for ^{68}Ga-RM2, RM2 (DOTA-4-amino-1-carboxymethylpiperidine-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH_2) is provided by Piramal Life Sciences, Imaging Division (Berlin, Germany, http://www.piramal.com). Radiolabeling will be performed in the Radiochemistry & Molecular Imaging Probes (RMIP) Core Facility at MSKCC. A Letter of Authorization from Eckert & Ziegler Eurotope, granting FDA access to the Drug Master File (DMF) for the Ga- generator is provided in section 7 of this IND.

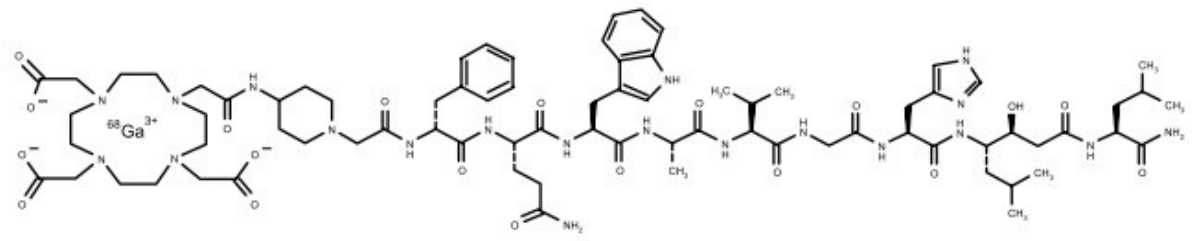


Figure 1. Chemical structure of ^{68}Ga-RM2

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

- Age ≥18 years
- Biopsy proven adenocarcinoma of the prostate
- Patients with low-risk, intermediate-risk and high-risk tumors according to NCCN guidelines (2.2014) will be included
- Planned radical prostatectomy at MSKCC
- Multiparametric MRI of the pelvis (performed or planned) as routine care

6.3 Subject Exclusion Criteria

Patients meeting any of the following exclusion criteria will not be eligible for study entry:

- Hematologic
  - Platelets <75K/mcL
  - ANC <1.0 K/mcL
- Hepatic laboratory values

  - Bilirubin >2.0 x ULN (institutional upper limits of normal)
  - AST/ALT >2.5 x ULN
- Renal laboratory values
  - Creatinine > 2.0 x ULN
- Claustrophobia interfering with MRI and PET/CT imaging
- Prior pelvic radiation
- Prior androgen deprivation therapy
- Patients deemed not surgical candidates due to prohibitive co-morbidities

7.0 RECRUITMENT PLAN

Potential study participants will be identified by a member of the patient's treatment team at MSKCC. Men of all races and ethnic groups will be considered for study participation. Candidates must conform to all inclusion and exclusion criteria to be accepted into the study.

If the investigator is a member of the treatment team, s/he will screen their patients' medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/ research staff of the study.

During the initial conversation between the investigator/ research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/ research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review.

8.1 PRETREATMENT EVALUATION

- History and physical examination
- Vital signs
- Laboratory studies (within 2 weeks before study entry):
  - Complete blood count
  - PSA
  - Serum BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, and albumin
- Prostate MR imaging

9.1 TREATMENT/INTERVENTION PLAN

Drug Accountability

The investigator is obliged to keep sufficient documentation of the delivery and use of the investigational product. The documentation will include administration date/ time, calibration date/time, quantity, patient code, lot number and date of destruction. The investigator should maintain records that document adequately that the patients were provided the dose specified in the protocol and reconcile the medication received for the study. The label of each vial will contain the patient code and lot number, patient and radiopharmaceutical information will be entered in the radiopharmacy drug accountability forms. The investigator may assign some or all of the investigator's duties for drug accountability to an appropriate individual such as pharmacist or nuclear medicine technician who is under the supervision of the investigator.

9.2 PET/CT imaging

PET/CT imaging will be performed within two weeks prior to the planned prostatectomy.

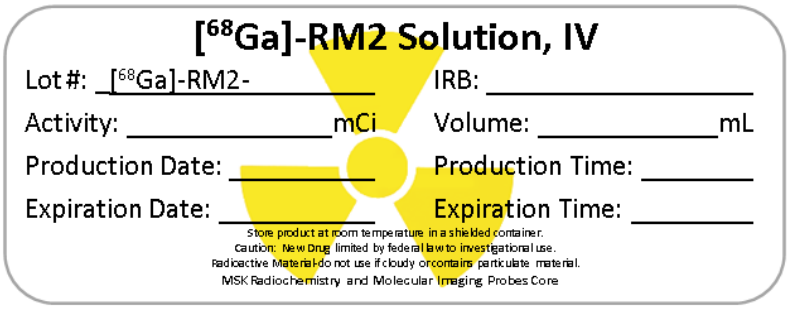
No specific patient preparation is required before ^{68}Ga-RM2 injection. One intravenous catheter (Hep-Lock) will be placed by staff in the Molecular Imaging and Therapy Service for radiopharmaceutical administration.

^{68}Ga-RM2 preparation

Radiolabeling of ^{68}Ga-RM2. Labeling will be performed in accordance with the IND. The study will be performed under an FDA approved IND for ^{68}Ga-RM2; no studies will be performed until the IND is approved. The final product will contain 150-200 MBq ^{68}Ga and a total mass of less than 40 µg RM2. The investigational product label will include the patient name, MRN and the calibration date and time. The investigational product is to be used within 60 minutes after manufacturing.

Package Labeling and Formulation

Each vial will contain the following information on the label:



Images will be corrected for attenuation and scatter and adjusted for system sensitivity and providing parametric images in terms of standardized uptake values (SUV) (= MBq found/gm tissue / (MBq injected/gm body mass).

PET scanning: In order to provide reproducible clinical data, we will acquire all ^{68}Ga RM2 PET scans on a PET-CT scanner at MSKCC (same or a newer generation scanner). Intravenous injection of ^{68}Ga-RM2 (maximum of 200 MBq) will be given as a bolus. A PET-

CT scan extending from top of skull to mid thighs will be performed to determine the biodistribution 60 min (+/- 10 min) after injection. The low dose CT portion of the study will utilize 80 mA.

If less than 150 MBq of ^{68}Ga-RM2 is acquired and injected, only a limited PET/CT will be performed over the pelvis and acquisition times per bed position will be increased.

Because the diagnostic accuracy of RM2-PET/CT for detection of prostate cancer and prostate cancer metastases is currently not known, the result of the RM2-PET/CT will in general have no impact on patient management. However, in the special case of focal RM2 uptake in the bone marrow (outside the field of view of the prostate MRI), a dedicated MRI of the involved region will be discussed with the referring clinician and the patient. Because bone marrow is a frequent site for prostate cancer metastases and studies in normal volunteers have not shown any relevant RM2 uptake in the bone marrow, focal RM2 uptake in the bone marrow has to be considered as suspicious for metastatic prostate cancer. MRI is highly sensitive and specific for bone marrow metastases and is not associated with an additional risk for the patient. If bone marrow metastases are seen on MRI, patients are generally no longer treated with radical prostatectomy. Because radical prostatectomy is associated with significant morbidity, we would therefore consider it as ethically problematic not to further evaluate focal RM2 uptake in the bone marrow by MRI.

9.3 PET/MR coregistration

Mirada (www.mirada-medical.com) provides dedicated software to permit manual rigid and deformable registrations to align images acquired in separate sessions. We have used this software to register PET and MR datasets through anatomical co-registration of the low-dose CT scan of the PET/CT and MR data. The resultant transformation was then applied to the PET data. This co-registration capability will allow us to meet our primary objective of determining the distribution of radiolabeled bombesin in the prostate within a —sextantǁ model used previously by our group (29). In this model the prostate is divided in left/right base, left/right midgland and left/right apex. Each of these sextants is further divided into peripheral and transition zones. We acknowledge the imperfect nature of the —sextantǁ approach for localization, particularly on PET/CT where the normal anatomy is less well-delineated, but co-registration with MRI based on anatomical landmarks such as the urethra, verumontanum and ejaculatory ducts should minimize localization errors and improve imaging to pathology correlation.

9.4 Immunohistochemistry and gene expression analysis

The expression of GRPr by prostate tumors will be assessed with immunohistochemical staining as previously described (30, 31). Preliminary data in prostate tumor xenografts treated with androgen deprivation therapy suggest that GRPr expression is AR regulated (32, 33). Conversely, GRPr expression can induce androgen independent growth of prostate cancer cells (34). We will therefore stain the tumors for nuclear AR as well as for PSA (positively regulated by AR) and PSMA (negatively regulated by AR, (35)) in order to interrogate the relationship between GRPr expression, *in vivo* ^{68}Ga-RM2 binding, and AR signaling. Semiquantitative assays for AR and PSA are established at the prostate SPORE core lab. The relationship of GRPr and PSMA expression is also interesting from an imaging

perspective, because radiolabeled PSMA inhibitors have shown encouraging results in metastatic prostate cancer (36-38). Staining for PSMA and GRPr will allow us to evaluate whether PSMA and GRPr based PET imaging provide complementary information about tumor biology. This is suggested by previous studies which found that Gleason scores are negatively correlated with GRPr expression but positively with PSMA expression (18, 39). A recent study indicated that GRPr is a shared target gene of two ETS transcription factors that are involved in the development and progression of prostate cancer (ERG and ETV1) (40). ETS rearrangements are considered an early event in prostate carcinogenesis, which may explain the overexpression of GRPr in PIN and low-grade prostate cancers. Nuclear expression of ERG is a sensitive and specific marker for the presence of the TMPRSS2-ERG rearrangement and ERG mRNA expression (41). We will therefore stain the tumors for nuclear ERG using established protocols at MSKCC (41) and correlate the staining with GRPr immunohistochemistry as well as *in vivo* ^{68}Ga-RM2 binding. Loss of PTEN is closely linked to aberrant gene expression by ERG transcription factor rearrangements (42). Staining for PTEN will therefore also be included in our analysis.

FISH analysis: In order to further investigate the relationship between ETS and AR signaling, we will use FISH for the TMPRSS2-ERG fusion gene (43) to assess the type of rearrangement (i.e., whether it is by deletion or translocation). We will also use FISH to assess the tumors for ETV1 rearrangements and AR amplification using established protocols at the prostate SPORE core lab.

Gene expression analysis: We will determine GRPr mRNA levels of the tumors, normal prostate and benign prostate tumors and correlate those with GRPr immunohistochemistry and *in vivo* uptake of ^{68}Ga-RM2. We will also determine the RNA expression levels for AR regulated genes including PSA, HK2 TMPRSS2, FKBP5, STEAP and PSMA as a readout for AR output. This —AR signature‖ will be correlated with uptake of ^{68}Ga-RM2.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Laboratory Safety Assessments

Hematology and blood chemistry assessments (CBC, PSA, serum BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, albumin, blood glucose levels, and serum testosterone level) will be done at the time points described in Table 2. Additional blood tests may also be performed at the discretion of the investigator for the purposes of investigation and follow-up of adverse events.

Physical examination

A physical examination including, but not limited to, general appearance, skin, neck, eyes, ears, throat, lungs, heart, abdomen, lymph nodes, extremities, and nervous system will be performed. The physical examination will include examination of known and suspected sites of disease. Physical examination results, height and weight will be recorded at baseline .

Vital Signs

Blood pressure and heart rate will be obtained at the time points outlined in the flowchart.

Table 2
Schedule of Assessments

	Screening		Day 1 (day of PET/CT)	Day 2 (after PET/CT)
	Within 4 weeks before PET/CT	Within 14 days before PET/CT		
Informed consent	X			
Demographics	X			
Medical history	X			
Concomitant medications	X			
Physical exam	X			
Performance status	X			
Vital signs	X		X	
Routine MRI	X			
Laboratory studies[1]		X		
Follow-up over phone				X
^{68}Ga-RM2 tracer administration			X	
^{68}Ga-RM2 PET/CT scan			X	

[1] CBC, PSA, serum BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, albumin, blood glucose, serum testosterone

Image data analysis

PET and MRI exams will be evaluated by a nuclear medicine physician (W. Weber) and an experienced radiologist (H.A. Vargas), respectively, blinded to the findings of the other imaging test, the biopsy results or any other clinical data except for the fact that the patient has prostate cancer.

PET/CT. The reader will record the level of suspicion for the presence of cancer in each sextant of the peripheral and transition zones using a 5-point index scale. The minimum intensity of uptake for a positive lesion (score > 1 on the 5 point scale) will be defined as mean uptake in normal prostate tissue plus two-times the standard deviation in prostate tissue without focal radiotracer uptake. Mean and maximum standardized uptake values will be recorded for each suspicious focus on PET. We will use ROC analysis to determine the sensitivity/specificity for prostate cancer detection by various visual scores and quantitative thresholds for lesion —positivity‖. Quantitative thresholds will be based on SUVs as well as lesion-to-background ratios. In addition to this analysis of the prostate, focal ^{68}Ga-RM2 uptake in lymph nodes, bones or other organs that is not explained by the normal biodistribution will be recorded.

MRI. Standard of care MRI studies at the Department of radiology are performed using a 3T whole-body unit. Transverse T1-weighted and transverse, coronal, and sagittal T2-weighted fast spin-echo sequences are obtained. Diffusion-weighted MRI is acquired in the transverse plane using a single shot spin-echo echo-planar imaging sequence with multiple b-values (b 0-1000). Dynamic contrast-enhanced MRI is performed using a transverse 3D T1-weighted spoiled gradient-echo sequence with a temporal resolution <7 seconds. Images are acquired after i.v. injection of 0.1 mmol/kg body weight gadolinium-DTPA at a rate of 2 mL/s.Based on these images the likelihood of prostate will be scored on a 5-point scale as described in several previous publication of the Department of Radiology, for example (29).

Joint analysis of PET and MR images. Following this independent reading of the MRI and PET images, a joint reading of PET and MR will be performed. This will allow us to localize the findings on PET to the prostate zonal anatomy on MRI. We will then also record the likelihood of cancer in each prostate region but now based on the combined information from the PET and MRI. This analysis is performed in order to evaluate if the combination of PET and MR imaging is more accurate than PET and MR alone. Since combined PET/MR scanners have recently been introduced it is clinically relevant to evaluate if the combination of ^{68}Ga-RM2-PET and MR is potentially more accurate for detection of prostate cancer than either ^{68}Ga-RM2-PET/CT or MR alone.

11.1 TOXICITIES/SIDE EFFECTS

Risks

^{68}Ga-RM2 PET/CT Scans

As part of this scan there is radiation delivered from the ^{68}Ga and from the low dose CT scan that are performed as part of the PET/CT for attenuation correction and co-registration. Although any exposure to ionizing radiation has the potential to cause some harm to tissue, the radiation exposures in this study are comparable to the low-level exposures associated with common diagnostic procedures such as CT scanning. There remains a low theoretical risk of developing a cancer at some point later in life as a result of the radiation exposure received in this study. This risk is much smaller than the clinical risks posed by the patient's current cancer. Participants should not father a baby while on this study. Acceptable birth control methods include abstinence, double barrier method, surgically sterilized patient or partner.

RM2

A recent study of ^{68}Ga-RM2 in 5 normal male volunteers reported no drug related adverse effects after administration of up to 28 µg ^{68}Ga-RM2 (26) . There were also no drug related adverse effects in a study of 14 patients with prostate cancer imaged with ^{68}Ga-RM2 (27). Other non-radioactive GRPr antagonists have been studied for treatment of prostate cancer and were well tolerated at several degrees of magnitude higher doses. In a phase I study the GRPr antagonists RC3095 was administered at doses ranging from 8-96 µg/kg once or twice daily, i.e. approximately 560 µg/day – 13440 µg/day. In this study the only discomfort observed was local discomfort at the injection site.

There is a potential small risk of infection at the site of injection.

Radiation Dosimetry

^{68}Ga-RM2 **Radiation Doses in the 70-kg Standard Man**

^{68}Ga	**Administered Activity:**	**200**	**MBq**
	# of 80-mA CT scans:	**1**	
	Organ dose per 80-mA CT scan:	**9**	**mSv**
			Absorbed Dose s

	^{68}Ga-RM2		^{68}Ga-RM2+CT
	mSv/MBq	mSv/scan	mSv
Adrenals	0.011	2.2	11.2
Brain	0.0056	1.12	10.12
Gallbladder Wall	0.011	1.2	10.2
LLI Wall	0.014	2.2	11.2
Small Intestine	0.01	2.8	11.8
Stomach Wall	0.038	2	11
ULI Wall	0.0094	7.6	16.6
Heart Wall	0.028	1.88	10.88
Kidneys	0.081	5.6	14.6
Liver	0.023	16.2	25.2
Lungs	0.0071	4.6	13.6
Muscle	0.0082	1.42	10.42
Pancreas	0.51	1.64	10.64
Red Marrow	0.013	102	111
Osteogenic Cells	0.013	2.6	11.6
Skin	0.022	2.6	11.6
Spleen	0.006	4.4	13.4
Testes	0.023	1.2	10.2
Thymus	0.01	4.6	13.6
Thyroid	0.007	2	11
Urinary Bladder Wall	0.027	122	131
Total Body	0.61	2	11
[redacted]			
Effective Dose (mSv/MBq)	0.051	10.2	19.2

Table 3. ^{68}Ga-RM2 absorbed Doses in 70-kg Standard Man per MIRD (OLINDA) Formalism Based on data from 5 volunteers. The calculations assume a bladder voiding interval of 3.5 h (26). Effective dose would be reduced by 25% by frequent bladder voiding (1h interval).

Assessment for Toxicity

Safety Outcome Measures (CTCAE v4)

Infusion Reaction

Patients will be monitored during and after ^{68}Ga-RM2 infusion for 120 minutes after the infusion. The safety and tolerability of ^{68}Ga-RM2 will be assessed using the following primary safety outcome measure:

- Incidence, nature, and severity of adverse events up to 1 day following peptide administration.
- Change in vital signs during administration

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Tumor response will not be assessed as part of the study.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Participation in the study is strictly voluntary. Patients have the right to withdraw from the study at any time. If a patient chooses to withdraw, he or she must inform the investigator immediately. In addition, the investigator has the right to terminate participation of any patient at any time if it is deemed in the patient‘s best interest. The reason and circumstances for premature discontinuation will be documented in the patient‘s medical records. Possible examples for reasons of premature study withdrawal include withdrawal of consent, SAE or intolerable AE, or any other medical illness at investigator‘s discretion.

14.0 BIOSTATISTICS

Accrual Rate

The Urology Service performs on average 600-700 radical prostatectomies per year of which patients with intermediate or high-risk disease represent the majority.This imaging study does not compete with therapeutic studies and there is also no competing imaging study for primary prostate cancer. Therefore, we expect an accrual rate of one patient per week.

Primary objective (specific aim 1)

The primary objective of this proposal is to estimate the sensitivity and specificity of PET using a gastrin releasing peptide receptor antagonist for localizing tumors of the prostate. Localization will be

defined using a total of 12 regions within the prostate: right and left base, midgland and apex in both peripheral and transition zones. The uptake on each region will be assessed by a radiologist blinded to standard of care imaging and clinical characteristics. Histopathological confirmation will be used as the gold standard.

Unit of analysis will be region and the correlation between regions of the same prostate will be handled statistically using a variance inflation factor (VIF) as described before (44). Primary analysis will involve an ROC curve for the uptake and calculation of the area under it. We will also report sensitivity and specificity in selected operating points from the ROC curve. With 20 patients and a total of 240 regions we will be able to estimate the sensitivity to within approximately +/- 11.4% and specificity to within +/- 8.1% with 95% confidence. These calculations are informed by a recent investigation of the use of 3T DW-MR imaging for the same disease at our institution (29). Specifically we used presumed values of 65% and 80% for sensitivity and specificity respectively, a VIF of 1.22 and a region-based prevalence of 40% which corresponds to approximately 100 tumor-bearing regions.

Secondary objective 1 (specific aim 2)

We will construct ROC curves separately for PET and MR, based on blinded five-point scale readings using histopathology as the gold standard. Curves will be compared using the Obuchowski method adjusted for clustering. We will also dichotomize the readings (0 and 1 as negative, and 2 or up as positive) and compare sensitivity and specificity of standard of care MR imaging with PET using a McNemar's test adjusted for multiple regions. The same comparisons will also be made for the joint analysis of PET and MR imaging vs. PET alone and MR imaging alone, respectively.

With 20 patients contributing 12 regions each we will have approximately 80% power to detect an absolute difference of 10% (e.g. 65% vs 75%) between the sensitivities and specificities (45) while controlling the Type I error rate at 5%.

Secondary objective 2 (specific aim 2)

Because of the limited knowledge on the relationship between uptake of ^{68}Ga-RM2 on the hand and AR signaling and ETS rearrangements on the other hand, we will perform descriptive, hypothesis generating analyses for this aim. Specifically, we will determine the ^{68}Ga-RM2 SUVs for tumors with and without ETS rearrangement, AR amplification, AR gene expression signature, and PTEN loss in a descriptive fashion. We will also study the relationship between SUVs and semiquantitative immunohistochemical parameters, such as GRPr and nuclear AR expression levels using Spearman rank correlation.

Exploratory analyses

Detection of lymph node metastases

The frequency of lymph node and distant metastases in the studied patient population is expected to be small (a total of 20 patients will be studied, only 6 of whom will be high risk patients). Therefore, sensitivity and specificity for detection metastatic disease will only be recorded in a descriptive way and not further analyzed.

Safety and tolerability of ^{68}Ga-RM2

The safety and tolerability of ^{68}Ga-RM2 will be assessed using the following primary safety outcome measures: Incidence and nature of DLTs; incidence, nature, and severity of adverse events; and change in vital signs and clinical laboratory results. Incidence and severity of DLTs and adverse events will be estimated with sample proportions and 95% confidence intervals while changes in vital signs and laboratory parameters will be tested using the signed-ranks test and summarized with descriptive statistics.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.3 Randomization

This research study does not require randomization procedures.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

Study personnel will record clinical data in each patient‘s source documents (i.e., the patient‘s medical record).

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the data stored in the database used for data collection. Records will be retained and securely stored for a minimum of 2 years after the completion of all study activities.

Data will be entered throughout the duration of the trial as patients are enrolled. Accrual is expected to last 2 years.

16.2 Quality Assurance

Regularly scheduled registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the principal investigator for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team at least once a year, more frequently if indicated.

16.3 Data and Safety Monitoring

The data and safety monitoring (DSM) plans at MSKCC were approved by the National Cancer Institute in September 2001. The plans address the policies set forth by the NCI in the document entitled *Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials*, which can be found at http://cancertrials.nci.nih.gov/clinicaltrials. The DSM plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC DSM plans can be found on the MSKCC Intranet at: http://mskweb5.mskcc.org/intranet/html/70775.cfm.

There are several different mechanisms by which clinical trials are monitored for data, safety, and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research quality assurance) and departmental procedures for quality control, and there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the MSKCC Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

This study will be conducted in compliance with the protocol, GCP guidelines established by the International Conference on Harmonization, and the ethical standards set forth in the Declaration of Helsinki 2004 (available at: www.laakariliitto.fi/e/ethics/helsinki.html).

The proposed patient studies are purely diagnostic in nature and, based on our extensive clinical experience with diagnostic radiotracers in general and diagnostic radiolabeled peptides in particular, will involve administration of the labeled peptide at mass and activity levels far below those which would induce any toxic or other pharmacological effect. However, as with any exposure to radiation, even at the diagnostic levels to be used in this study, there is a very low, theoretical risk of radiation-induced cancer at some later point in life. The projected normal-organ radiation doses to subjects in this study are determined and included in our protocols and are well in the range of typical diagnostic imaging tests for cancer patients, such as CT. The risk of subsequent radiation-induced cancer is explicitly included in the Informed Consent forms for the studies included in this protocol. In addition, the subjects who will be enrolled in this clinical investigation will be exclusively patients with prostate. The additional radiation doses associated with the PET or PET/CT scans involved in this study, will typically represent a small increment in their "lifetime" exposures. The typical age of patients with prostate cancer will also decrease the likelihood for radiation induced cancer.

This protocol does not have therapeutic intent and does not offer patients therapeutic benefit. This will be clearly conveyed to patients when communicating the potential toxicities/side effects of participating in this trial. Participation in the trial is voluntary and there will be no financial benefit (or burden) for the patients. Participants will not be charged for ^{68}Ga-RM2 tracer injections, ^{68}Ga-RM2 PET/CT scans, and research testing on tumor tissue obtained from prostatectomy.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 _Reporting of Serious Adverse Events', the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1

This protocol will have an IND. SAE will also be reported to the FDA through the IND office and the report will include the FDA assigned IND number and name.

17.3 SAE Recording and Grading

17.3.1 Recording

The investigator must assess each event to determine if it meets the criteria for classification as a serious adverse event (SAE) or serious adverse drug reaction (ADR). An SAE/ADR as defined in the Code of Federal Regulations (21CFR312.32) is any event that:

- Results in death
- Is life-threatening
- Results in inpatient hospitalization or prolongs an existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in congenital anomaly/birth defect
- Is medically significant in the opinion of the investigator

All SAEs that occur any time a patient is on study (i.e., as soon as the informed consent has been signed) or within 30 days of the ^{68}Ga-RM2 PET/CT scan must be reported, regardless of the suspected relationship to the study drug. Any SAE occurring more than 30 days after the PET/CT scan must be reported if a causal relationship is suspected.

When accounting for many tests per year, the radiation doses (on the order of 20 mSv) lie well within the —low-dose‖ range—well below the threshold doses (typically on the order of 1000 mSv or greater) for any known deterministic effects. A detailed description of the expected dosimetry from the ^{68}Ga-RM2 has been published. The dose of ^{68}Ga-RM2 per administration (150-200 MBq) gives the lowest amount that would be consistent with the research goal. Lower doses of radioactivity would limit image quality and will limit the detectably of prostate cancer on PET.

17.3.2 Grading Severity

All adverse events will be graded for intensity on a scale of 0 to 5. Severity grades will be recorded and based on the CTCAE v4.0.

17.3.3 Attributing Causality

The investigator will evaluate the potential relationship between all clinical AEs, abnormal laboratory values, and the ^{68}Ga-RM2, and categorize the relationship according to the descriptions in Table 5. Abnormal laboratory values of clinical significance that were present at baseline and did not change in either severity or frequency during the experimental therapy or intervention and/or that can obviously be attributed to the underlying disease will be evaluated by the investigator and recorded in the —unrelated‖ category.

Table 6. Relationship of Adverse Event to ^{68}Ga-RM2

Relationship	Description
Unrelated	AE is clearly not related to the ^{68}Ga-RM2
Unlikely	AE is unlikely related to the ^{68}Ga-RM2
Possible	AE may be related to the ^{68}Ga-RM2
Probable	AE is likely related to the ^{68}Ga-RM2
Definite	AE is clearly related to the ^{68}Ga-RM2

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

Not applicable