Thomas Jefferson University

# *THOMAS JEFFERSON UNIVERSITY*
# *Kimmel Cancer Center*

## A Pilot Phase I Open Label Study of Cu-64-TP3805 PET Imaging for Detection of Prostate Cancer in Men Persistently Elevated PSA

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## List of Abbreviations

| | |
|---|---|
| 3D | Three dimensional |
| BC | Breast cancer |
| BPH | Benign prostatic hyperplasia |
| C-11 | Carbon 11 |
| CT | Computed tomography |
| Cu-64 | Copper 64 |
| dL | deciliter |
| DOTA | 1, 4, 7, 10-tetraazadocdecane –N, N", N'", N"", -tetra acetic acid |
| F-18 | Fluoride 18 |
| FDG | Fluorodeoxyglucose |
| FLT | Fluorothymidine |
| hCtr1 | human copper transport 1 protein |
| IAS | Internal anal sphincter |
| IC50 | half maximal inhibitory concentration |
| IACUC | institutional animal care and use committee |
| ID/g | Injected dose per gram of tissue |
| In-111 | Indium 111 |
| kD | Dissociation constant |
| kDa | KiloDalton |
| MCF-7 | Michigan Cancer Foundation-7 breast cancer cell line |
| mCi | Millicurie |
| MR | Magnetic resonance |
| MRI | Magnetic resonance imaging |
| MW | Molecular weight |
| ng | Nanogram |
| nM | Nanomole |
| PACAP | Pituitary adenylate cyclase activating peptide |
| PC | Prostate cancer |
| PC-3 | Prostate cancer cell line |
| PET | Positron emission tomography |
| Phe | Phenylalanine |
| PSA | Prostate specific antigen |
| PSMA | Prostate specific membrane antigen |
| RTPCR | Reverse transcriptase polymerase chain reaction |
| SD | Standard deviation |
| Tc-99 | Technectium 99 |
| TRAMP | Transgenic adenocarcinoma of the Mouse Prostate |
| TRUS | Transrectal ultrasound |
| Tyr | Tyrosine |
| VIP | Vasoactive intestinal peptide |
| $VPAC_1$ | Vasoactive intestinal polypeptide receptor 1 |
| DCE-MRI | Dynamic contrast enhanced MRI |

**Study Summary**

| | |
|---|---|
| Title | VPAC1 Receptor Targeted PET Imaging of Prostate Cancer (PC) |
| Short Title | PET Imaging of PC |
| Protocol Number | 15F.264 |
| Phase | Pilot Study (Phase I, feasibility study) |
| Methodology/Study Design | Single Arm, Open Label Diagnostic Study |
| Study Duration | 3 years |
| Study Center(s) | Single-center. |
| Objectives | The objective is to validate the PET imaging results of Cu-64-TP3805, with the histology of the image guided biopsy results. Generally, approximately 60% of the patients with elevated PSA have prostate cancer |
| Number of Subjects | 20 |
| Diagnosis and Main Inclusion Criteria | We will study 20 patients with persistently elevated PSA whom are scheduled for biopsy.. These patients shall be PET imaged with our Cu-64 peptide probe first and then a week or so later the image guided biopsy shall be performed. The image guided biopsy is the standard of care procedure. The objective is to validate the PET imaging results with the histology of biopsy results. Generally, approximately 60% of the patients with elevated PSA have prostate cancer. We expect 80% accuracy in identifying 20 subjects as having histologically determined cancer/absence of cancer. The 95% confidence interval of this level of accuracy for this sample size is + or – 17.5%. Thus we expect to find an accuracy level between 62.5% to 97.5%. Please note this is a pilot study, not designed to be an efficacy determination trial. |
| Study Therapy, Dose, Route, Regimen | Cu-64-TP3805, a novel PET imaging molecule, given as a single intravenous injection dose of (4 mCi ±10%). |
| Duration of administration and follow-up | Single Intravenous administration given over 5 minutes and patients observed for 90 min; follow up, call 24 hrs later. |
| Reference therapy | N/A |

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| Statistical Methodology | We expect 80% accuracy in identifying 20 subjects as having histologically determined cancer/absence of cancer. The 95% confidence interval of this level of accuracy for this sample size is + or – 17.5%. Thus we expect to find an accuracy level between 62.5% to 97.5%. Please note this is a pilot study, not designed to be an efficacy determination trial. |
| Schema | Subjects will undergo Cu-64-TP3805 PET Imaging at least one week prior to biopsy. Investigational PET imaging will be analyzed and compared to biopsy pathology to determine efficacy in detection and localization of PC foci. Urine sample will be collected prior to Cu-64 injection and 90 minutes post injection prior to imaging |

## 1.0 INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

### 1.1 Specific Aims and Hypothesis

We hypothesize that targeting VPAC1 receptors overexpressed on PC cell surfaces with a Cu-64 imaging agent, TP3805 will provide a specific and sensitive means for molecular diagnosis of PC at an early stage, to determine its recurrence, and to image metastases.

Our specific aim is to assess the ability of Cu-64-TP3805 to detect PC within the prostate gland.

### 1.2 Background and Rationale

Cancer is complex yet commonplace, and the most terrifying disease of mankind. Among men in the USA, PC is particularly lethal, second only to cancers of the lung and bronchus combined [1]. In 2010, more than 30,000 men succumbed to PC and more than 240,000 new PC cases were identified in the USA [1]. PC affects one in every 6 men who are 60 years or older and affects African-Americans at a rate 2.4 times greater than European-Americans [1]. Great strides have been made both in diagnosis and treatment of PC. However, the PC-related death rate has not yet declined, and the quality of life of survivors has not yet improved [1]. Three prominent screening tests, the digital rectal examination, MRI, and a blood test for prostate specific antigen (PSA) determination play a significant role in detecting pathologically advanced PC. However, they are not considered reliable tools for early warning of PC [2], [3], [4] nor can they be used reliably to detect recurrent cancer or to determine

metastatic status of the disease. The lack of reliable diagnosis, results in undertreatment or overtreatment of patients with minimal benefit, enormous morbidity, incontinence, and/or impotence. Therefore, histology remains the mainstay of PC confirmation. However, out of >750,000 biopsies performed each year in the USA, >65% show benign pathology, and cost hundreds of millions of health care dollars. Various imaging techniques have been evaluated, but their utility to image suspected PC or its metastatic lesions is not fully dependable [3]. Furthermore, monitoring the effectiveness of PC therapy continues to be a challenge.

Our proposed approach to image PC, its metastases, and recurrence is driven by targeting an endogenous genetic product overexpressed when cells suffer genetic damage that ignites cancerous transformation. These characteristic fingerprints, the VPAC1 cell surface receptors, express themselves at the onset of the malignancy, and may be prior to elevation of PSA, and well before cell morphology is altered [5]. Thus VPAC1 receptors provide a specific target that has not yet been exploited for imaging suspected PC and its metastases [5]. VPAC1 mediates VIP (vasoactive intestinal peptide) and PACAP (pituitary adenylate cyclase activating peptide) growth hormone function in all types of PC [6-8]. During the past 10 years we have successfully initiated the use of Cu-64 labeled VPAC1 receptor-specific peptide constructs to image disease specific oncogene products in experimental animal models of pancreatic, prostate and breast cancer (BC), and in humans with BC [9-13].

We hypothesize that targeting VPAC1 receptors overexpressed on PC cell surfaces [6-8] with a pair of Cu-64 imaging agents, TP3939 (a VIP analog) or TP3805 (a PACAP analog), will provide a specific and sensitive means for molecular diagnosis of PC at an early stage, to determine its recurrence, and to image metastases. This molecular approach for diagnosis is both novel and feasible for deployment in the clinic. Furthermore, it promises to reduce the need for invasive biopsies, and meet the compelling need to reduce overtreatment, and surgery yielding minimal benefit, incontinence, or impotence. Importantly, PET imaging costs much less than biopsy combined with histology [14-16].

The need for developing oncogene receptor specific agents, such as those proposed here, is compelling. The primary tools for PC screening, the serum PSA and the digital rectal examination are neither specific nor sensitive, as many men with elevated PSA have been found not to have PC [2]. Transrectal ultrasonography (TRUS) provides an axial and sagital examination of the prostate gland, and allows physicians to estimate its volume. However, in 60-70% of PC cases are hypoechoic and thus invisible by TRUS [3]. Histologic examination, therefore, remains the gold standard for PC but requires an invasive procedure for tissue sampling. By this time, the PC is usually advanced. However, the question still remains as to where to direct the needle for biopsy. As a result, out of the approximately 750,000 prostate biopsies that will be performed in the USA in 2010, more than 2/3 will find benign pathology [17].

The use of contrast enhanced spiral computerized x-ray tomography (CT) is limited to advanced, high-grade tumors [18]. Using 3D MR spectroscopic imaging, PC was diagnosed as possible if the ratio of choline to citrate exceeded 2SD above the normal population and definite if the ratio exceeded 3SD above the normal population [19]. However, sensitivity and specificity were only 80% and 46%, respectively [20]. Dynamic contrast enhanced MRI (DCE-MRI) is known to be a powerful tool for localization of vascular solid tumors [21].

DCE-MRI however has low sensitivity (50%) [22] and has not been able to distinguish between PC and benign prostatic hyperplasia (BPH) [23]. T2 weighted endorectal MRI also has low accuracy of PC detection [24]. Much more work therefore, is needed to be done [25].

Agents for scintigraphic molecular imaging of PC are F-18-FDG, In-111-ProstaScint [26], C-11-choline, C-11-acetate [18], F-18-FLT (F-18-Fluorothymidine) and F-18-Fluorocholine. Cu-64 Bombesin [27], 2-F-18 fluoropropianic acid [28] and those target PSMA [18]. Although C-11-acetate and C-11-choline may have improved scintigraphic advantage over F-18-FDG imaging, their efficacy in use centers is lacking. The F-18-FDG uptake is high in bladder, low in PC and overlaps with uptake in the normal gland, postoperative scar or local recurrence. These complicate the visualization for PC imaging even further [29]. Similarly the low tumor/blood ratios and high bladder uptake, of 2-F-18-fluoropropianic acid, may question its utility in humans [28]. In-111-ProstaScint or Tc-99m and PSMA antibody have not been considered to be a useful agent [18]. Despite these weaknesses, there have been 195 investigations in which F-18-FDG was employed and additional 109 in which In-111-ProstaScint was utilized (PubMed, 2012). Generally, these agents were used for imaging primary and recurrent disease, metastatic disease and to determine the influence of therapy of the PC. In none of the cases did the agents work with reliability, high sensitivity, or specificity. These investigations demonstrate a compelling need for imaging agent that can help physicians in the management of desperate PC patients. Genetic imaging can provide unique opportunities not only for imaging PC in suspected PC patients, but also for evaluating treatment response [13, 30].

With Cu-64-Bombesin tumor uptake was only 1.32±0.49% ID/g at 4 hr and 0.28±0.21% ID/g at 24 hrs, which is significantly lower than the 7.5±3.6% ID/g at 4 hr uptake with one of our Cu-64 labeled VPAC1 specific peptide analogues, TP3939, that did not significantly decline as a function of time [27, 30]. We used the same animal model and the same cell line as those in the Cu-64 Bombesin protocol. Additionally, the steady uptake of Cu-64-TP-3939 makes sense as more of the probe continued to bind to the VPAC receptors (see later). In contrast, the declining and low uptake of Cu-64-Bombesin uptake was puzzling.

Ionic Cu-64-$Cl_2$ has been shown to be taken up by PC (3.0±0.7% ID/g) [31], via human copper transport 1 protein (hCtr1). As can be seen in Table-2, the tumor uptake of Cu-64-TP3939 in the same PC-3 human PC tumors, in athymic nude mice, was 7.48±3.63 %ID/g. The Cu-64-$Cl_2$ tumor uptake in the

same model in our laboratory was 4.79±0.34 %ID/g, consistent with data reported by the investigators referenced above. Assuming that the hCtr1 gene will carry Cu-64-$Cl_2$ to MCF-7 human BC, which overexpresses VPAC1 receptors in nude mice, we repeated the study in MCF-7 model and found that the tumor uptake was <2% ID/g. This suggested that the uptake of Cu-64-$Cl_2$ may be dependent upon the tumor vascularity. In order to further elucidate the nature of Cu-64-$Cl_2$ tumor uptake, we performed cell binding assays using ionic Cu-64-$Cl_2$ with PC-3 cells. The data were analyzed as per Scatchard, strongly indicate that the binding was not related to any specific mechanism (Preliminary Results). These findings suggest that Cu-64-$Cl_2$ for PC imaging cannot be reliable. The need for better agents still persists.

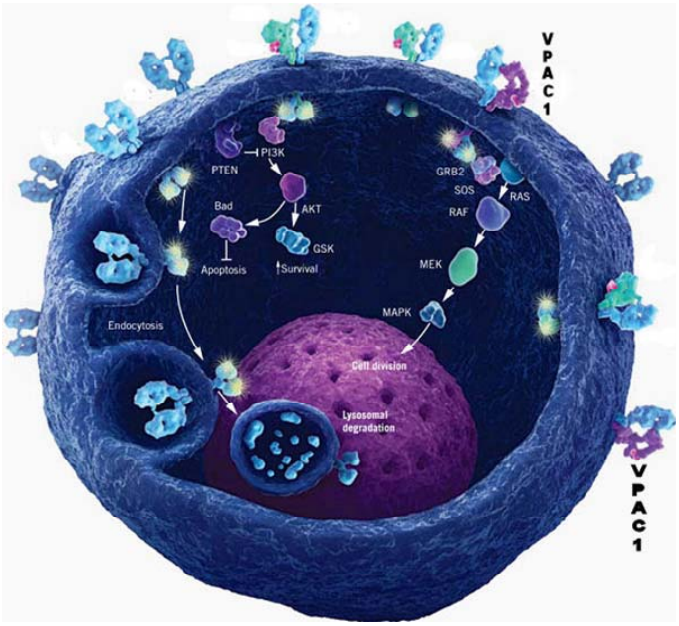

Fig. 1. VPAC1 receptor activates prostate cancer cell proliferation

Rapidly evolving advances in genomics and proteomics shed new light on the genesis of many diseases every day and provide us with new approaches in molecular imaging, to diagnose and treat them. Fig. 1 is the schematic presentation that illustrates cell signaling pathways that overexpress specific fingerprints at the onset of a malignant disease within the cell nucleus or cytoplasm and on the cell surface. Little or no attempts have yet been made to utilize these databases to image PC. In this investigation, we chose to target the well characterized, overexpressed VPAC1 surface receptor in human PC.

Our early data in xenografts, transgenic mice that spontaneously grow PC, and mimic pathogenesis of human PC are promising, and so are the early results in PET imaging of human BC in which we have also targeted VPAC1 receptors in nineteen patients.

## Background

### VPAC1 and VPAC2 Overexpression:

Reubi and colleagues [6-8] examined more than 600 tumors and their metastases using histochemistry and conclusively reported that VPAC1 and VPAC2 receptors are overexpressed on a variety of frequently occurring human tumors including those of the breast, prostate. On 100% of the human prostate tumors examined (n=35), VPAC1 receptors were predominantly overexpressed

on PC tissues and VPAC2 on stroma, to a lesser extent. Although VPAC1 receptors exist on normal cells, their expression is lower on normal than on malignant cells [32, 33] on which the receptor density is high ($10^4$/cell) [34, 35]. A 28 amino acid peptide VIP has high affinity for VIP receptors and the 27 amino acid peptide PACAP have high affinity for VIP and PACAP1 combined oncogene receptors expressed on malignant cell surface. Therefore, we hypothesize that radiolabeled VIP and PACAP1 or their analogues will provide us with excellent compounds for accurate and sensitive detection of human PC, once PC is suspected. The probe can also detect metastases, and determine therapeutic effectiveness. This will bridge the large void that exists today.

**Why Investigate VPAC1 Biomarker? Why Cu-64? And Why PET?**

The implications of diagnosis by receptor-specific imaging in the management of PC, and cancer in general, cannot be overemphasized [5]. If successful, the VPAC1-targeted peptides are will provide a specific and sensitive means for a true, early diagnosis in all patients suspected of having PC, and a guide to the most appropriate therapy. It can detect metastatic lesions and distinguish PC from benign masses. We have targeted VPAC1 receptors which are expressed also on human (BC). and have achieved success in PET imaging of BC in nineteen humans (Fig. 2a, Fig. 2b). Our studies in a transgenic (MMTVneu) mouse model that spontaneously grow BC, and overexpress VPAC1 receptors, demonstrated that Cu-64-TP3805 correctly imaged all eight unknown BC and its metastases. All eight tumors expressed VPAC1 receptors (RTPCR). Cu-64-TP3805 did not image two benign

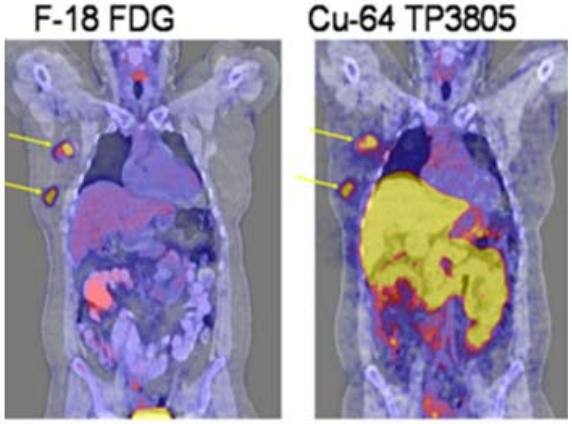


**Fig. 2a.** A 39 yr old Caucasian female with no family history but with two palpable lumps and suspicious mammogram and positive histology had a positive F-18-FDG scan (arrows) a week prior to Cu-64-TP3805 PET scan. Both lesions (arrows) including a pair of involved lymphnodes, consistent with the F-18-FDG scan are delineated.

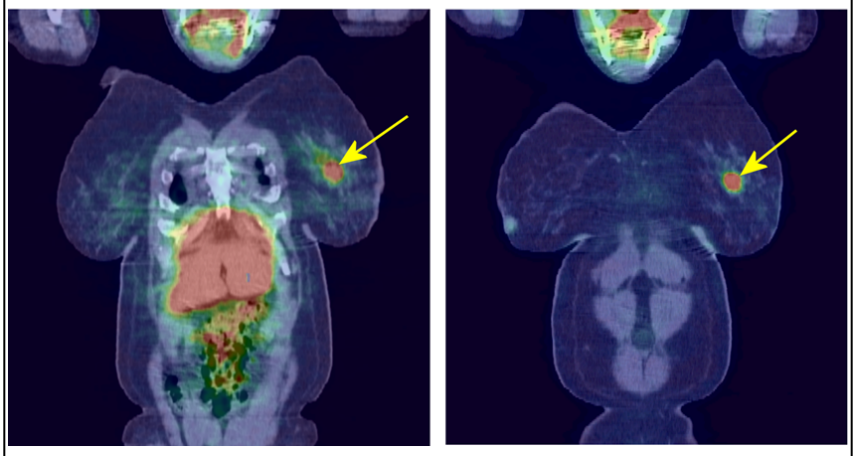


**Fig.2b.** A 43 yr old female with positive histology for BC and positive F-18-FDG scan (right, arrow) was given 2.2 mCi Cu-64-TP3805. A PET/CT fusion image of Cu-64-TP3805 (left, arrow), shows the same lesion clearly delineated. (Once the feasibility of Cu-64-TP3805 to image BC is established in first group of patients (n=6). We will use Cu-64-TP3805 to image patients (n=19), with suspicious mammography. Histology will confirm or deny malignancy and F-18-FDG image will be obtained for comparison. This will allow us to determine the ability of Cu-64 genetic imaging to distinguish between malignant and benign lesions).

lesions which did not express VPAC1 receptors and demonstrated superb specificity [36]. Our Preliminary results in imaging PC also show reasonable, hypothesis-driven, success both in experimental human PC grown in immunocompramised mice as well as spontaneously arising PC in TRAMP mice. VPAC1 surface biomarkers are a straightforward target [27, 37].

These data attest three important parameters as they pertain to this proposal: i) That the BC imaging data generated in mice by targeting VPAC1 are reliable and provide a firm foundation for translation into humans to image BC. ii) The PI and his team have the appropriate expertise and ability to drive basic research into clinical applications and iii) These data strongly support our hypothesis that the proposed investigation will enable us to initiate studies in patients suspected to have PC or are suffering from PC. BC PET imaging either with F-18 VIP analogues or Tc-99m-VIP analogues was less successful than that with Cu-64 VIP/PACAP analogues [37-39]. Copper chemistry is well known and Cu-64 (t ½ 12.8 hr) half-life is long enough to obtain commercially and use in humans conveniently. Position emission from Cu-64 is 19%.

The relatively low (4-6 mm) spatial resolution of current human PET scanners may be a problem. However, our tumor uptake/g with 4±10% mCi Cu-64/injection will be 240 µCi (6% ID/g) for ~7 µCi in 20 mg, a 1.7 mm diameter tumor. This amount of radioactivity will be large enough for unequivocal delineation. We further anticipate that the continuous reassessment and refinement in PET technique will also add to its improved resolution in years to come [40, 41]. The Cu-64 PET images with Cu-64 TP3805 promise early, accurate, and specific BC images, as we have seen in humans (Fig. 2a, Fig. 2b) by targeting VPAC1 receptors with SUV of 9-11. Cu-64-TP3805 unequivocally detected twenty out of twenty malignant lesions in nineteen patients we have studied so far. Additionally, four involved lymph nodes were also delineated. (Please see attached manuscript (J Nucl Med, 54, No. 7, 2013). Because the prostate is anatomically distant from the liver, we expect that PC lesions will be distinct despite the relatively high liver uptake. Furthermore the lack of bladder uptake facilitates PC delineation (please see later, Fig. 9). We further anticipate that metastatic bone lesions in PC patients will also be readily discerned by PET imaging.

**Innovation:**

We have designed, synthesized and characterized specific probes to target VPAC1, labeled them with Tc-99m for SPECT and with Cu-64 for PET imaging of BC and PC (Preliminary Results, below). This molecular approach targets a specific biomarker, discovered through modern advances in genomics and proteomics. Their translation to PC patients is a natural and timely progression into a new clinical capability. Our earlier results, including human BC imaging, are highly encouraging [9, 38]. These results lead us to hypothesize that one or more of the VPAC1-specific peptides will provide us with a tool for sensitive and specific PET imaging of suspected primary and metastatic PC

lesions. We expect that VPAC1 receptor specificity will differentiate malignant lesions from benign masses noninvasively, Unequivocal identification of PC lesions could help physicians tailor an appropriate therapeutic intervention. The anticipated reduction in false positives and false negatives should reduce morbidity and mortality, and improve the quality of life of PC patients. This novel approach to image PC by targeting a gene product is hypothesis-driven and based upon our own experience and the wealth of information yielded by recent advances in genomics and proteomics. When prostate cells undergo genomic modulations for whatever reason, they overexpress VPAC1 cell surface receptors. These changes occur well ahead of shed PSA or cell-morphologic alterations, the histology of which forms the basis of current PC diagnosis. The specificity of genomic biomarkers, should permit us to image PC in patients.

Biological fluids, including urine, represent a promising source of biomarkers for detection and prediction of PC prognosis. Because urine is available non-invasively and readily, numerous studies targeting DNA, RNA, protein and metabolite based biomarkers have been performed. However, none have yet reached the clinic]. Even FDA approved PCA3 test has low sensitivity and limitations in predicting aggressive PC .

VPAC1 cell surface genomic receptors are over-expressed at the onset of the malignancy, prior to elevation of PSA, and well before cell morphology is altered . We hypothesized that VPAC1 receptors expressed in high density on PC can be targeted for detection of shed tumor cells (STC) in patient urine, using TP4303, a VPAC1 specific biomolecule labeled with a near infrared fluorophore.

The success of these goals will meet the compelling need to a) localize primary PC, that can guide biopsy procedures. b) detect recurrent disease and c) image metastatic lesions with improved reliability. Reliable detection of PC can reduce the need for invasive biopsies, and minimize overtreatment, including surgery. It is important to note that overtreatment can cause incontinence or impotence. Thus, VPAC1 imaging addresses serious healthcare issues that current approaches cannot resolve, despite the great strides made in recent decades.

The results of our previous translational research and early applications in imaging BC in humans are published [11, 13, 42, 43] [35-38, 44] [36]. Our studies in a transgenic (MMTV-neu) mouse model that spontaneously grow BC, and overexpress VPAC1 receptors demonstrate that Cu-64-TP3805 correctly imaged all

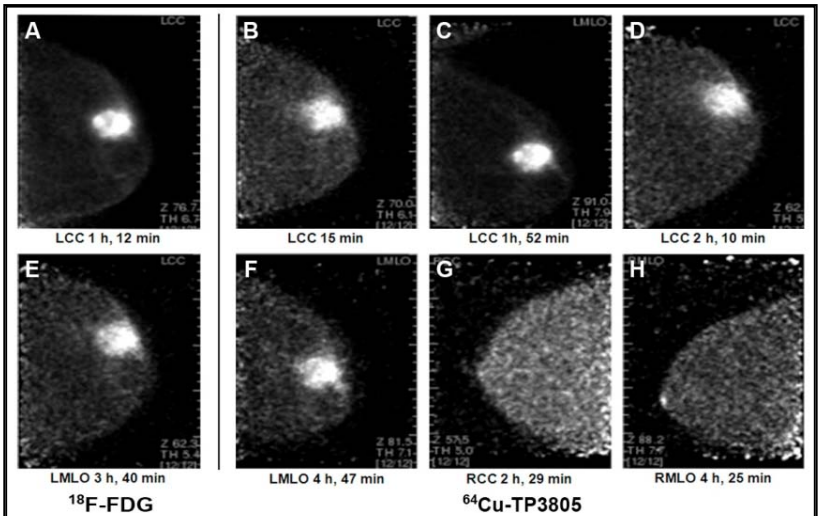


**Fig. 2.c**. F-18-FDG (A, 370 MBq, (10 mCi) and Cu-64-TP3805 (B, 159.1 MBq, (4.3 mCi), F-18-FDG equivalent 860 µCi) PEM scans of the left breast of a 39 yr old female. The F-18-FDG scan was obtained 1 hr post injection and the Cu-64-TP3805 scan was acquired 15 min post injection. The PUV/BGV ratios for the lesion (IDC, ER+, PR+, $HER_2$-) were 2.9 for F-18-FDG and 2.2 (lowest of the series) for Cu-64-TP3805.

eight unknown BC and its metastases. All eight tumors expressed VPAC1 receptors (RT-PCR). It did not image two benign lesions that did not express VPAC1 receptors and demonstrated superb specificity [36]. Our early results in imaging human BC are also promising, (Fig. 2.a, 2.b and 2.c). This success is both assuring and stimulating and promises similar success in PC imaging.

### 1.3 Study Therapy

This is a diagnostic product developed by the PI to specifically target genomic biomarker VPAC1 that is expressed on breast and PC cells in high density. With FDA approved eIND (101550) and all institutional approvals, the PI has used this radiopharmaceutical Cu-64-TP3805 in nineteen human subjects with BC. The sensitivity of this agent to image BC lesions and sentinel nodes was 100%.

This project for imaging PC will use the same agent, Cu-64-TP3805 for which the approval by FDA is obtained.

### 1.4 Preclinical Data

#### 1.4.1 Vasoactive Intestinal Peptide (VIP) and its Analogues:

VPAC1 receptors are overexpressed on all PC, including metastatic lesions (Fig. 3) [6, 8, 45]. High expression of VPAC1 receptors (>$10^4$/cell) has been observed by others [34, 35, 39]. Both VIP28 and PACAP27 have high affinity for VPAC (VPAC1 and VPAC2) receptors. VIP is a 28-amino acid peptide (Fig.4) initially isolated from porcine intestine [46]. VIP, whose structure is common in humans, pigs and rats, is a hydrophobic, basic peptide containing three lysine [18, 24, 25] and two arginine [17, 45] residues. From the essential histidine residue at the N-terminus to the amidated C-terminus, all 28 amino acids of VIP are required for high affinity binding and biological activity [47].

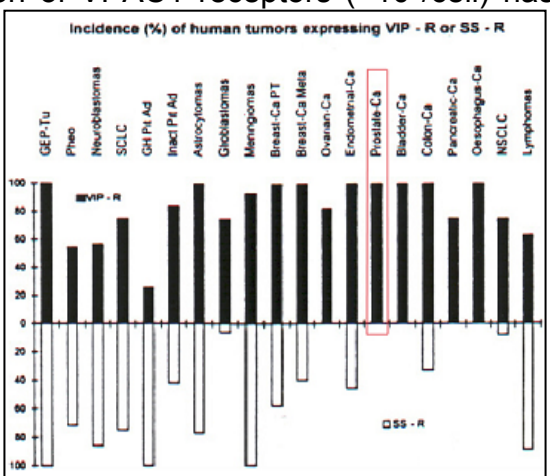


**Fig. 3**. Incidence (%) of human tumors expressing VIP (now classified as VPAC1, Ref.6) receptors (in comparison

VIP gene receptors (VIP1 and VIP2) have been detected on the cell membrane of normal intestinal [48] and bronchial epithelial cells [48, 49] and are overexpressed on various cancer cells, including colonic adenocarcinoma [50-53], pancreatic carcinoma [53] and cancers of the prostate [6, 8, 45]. VIP (Tyr10 and Tyr22) labeled with I-123 successfully imaged a number of human

$NH_2$-His-Ser-Asp-Ala-Val-Phe-Thr-
Asp-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-
Gln-Nle-Ala-Val-Lys-Lys-3-$OCH_3$-4OH-Phe
Lue-Asn-Ser-Val-Lue-Thr-
yAba- Lys- COOH

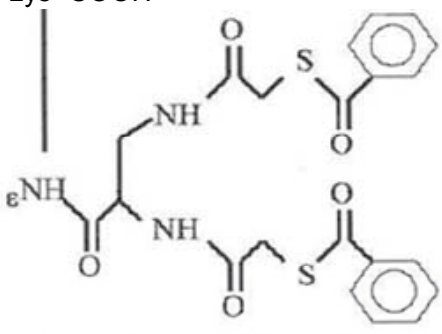


**Fig. 4.** Schematic presentation of TP3939

tumors [32]. Following PACAP homology, these gene receptors are recently named VPAC1 (for VIP1 and PACAP2 combined) and VPAC2 (for VIP2 and PACAP3 combined).

VPAC1 receptors are internalized after binding to VIP28 [39]. Imaging results of F-18-VIP have not been encouraging [35, 39]. Because VPAC1 receptor density is greater in malignant tissue than in benign tissue, tumors can be imaged with greater contrast relative to normal tissue [7, 39, 54].

Two analogues of VIP28 with several-fold greater potency have been identified [55]. We have developed methods to include chelators (Fig.4) to bind a single Tc-99m and Cu-64 cation without compromising VIP biological activity. Each PC cell has ~$10^4$ receptors and each cm3 tumor includes ~109 cells, amounting ~1013 receptors/cm3 tumor. Assuming, conservatively, that only 5% of these receptors were occupied with Cu-64-VIP, each cm3 tumor would contain 5x1011 molecules of Cu-64-VIP, i.e., 200 µCi of Cu- 64. With high target to background ratios, a lesion containing 10 µCi Cu-64 can be unequivocally delineated by a human PET scanner. The tumor uptake with our Cu-64-TP3939 7.5% ID/g [30]. For a human dose of one mCi, one calculates 75 µCi Cu-64/g tumors. Up to 25 mCi Cu-64 probe (ATSM) has been administered to humans [56]. A tumor weighing <20 mg (1.7 mm diameter) can contain 4-5 µCi and be easily detectable particularly when the bladder and the normal stromal activity are negligible and when tumor/normal prostate radioactivity ratios averaged nearly 4 at 4 hr post injection and 2.7 at 24 hr post injection [30]. Theoretically, this should be not only a specific, but also a sensitive technique to detect a relatively small number of malignant cells that cannot be detected non-invasively by any other technique available today. These facts support our hypothesis that Cu-64-VIP analogues may serve as useful PET molecular probes to detect VPAC1 expression for early imaging of PC, its recurrence, metastatic lesions, and in long-term should also help physicians to determine effectiveness of therapy.

The fact that PACAP27 recognizes and has high affinity (kd=1.5 nM) for both VIP1 and PACAP (VPAC1) receptors that are overexpressed on PC cells suggests that PACAP may also be a suitable agent to image PC [8, 57-67].

Our data show that these Cu-64 probes are highly stable in vivo [30] [38]. Furthermore, the high VIP affinity for receptors on malignant cells and subsequent internalization [10, 11, 13, 30, 37, 43] [38] minimizes its proteolysis and allows cell detection, as we have demonstrated in both mice and humans [30, 36, 37]. We synthesized one analogue of VIP28 (TP3939) and one of PACAP27 (TP3805) that are more potent and biologically stable than VIP28. Their amino acid sequences are given in Table-1. These analogues have the high IC50 values (4.4 nM and 5.3

**Table 1**: Amino acid sequence of TP3805 and TP3939

| Analog | Sequence | Name |
|---|---|---|
| Custom Peptide (PACAP 27 analog) | His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-Ser-Arg-Tyr-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Ala-Ala-Val-Leu-Gaba-Lys (Dap-(BMA)2) | TP3805 |
| Custom Peptide (VIP28A analog) | His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Val-Lys-Lys-(3-OCH3,4-OH)-Phe-Leu-Asn-Ser-Val-Leu-Thr-Gaba-Lys(Dap-(BMA)2) | TP3939 |

nM, respectively) among the many that have been synthesized and evaluated [55, 67].

The rationale for choosing TP3939 VIP analogues was as follows. VIP28 is comprised of three aromatic moieties at Phe6, Tyr10, and Tyr22, a negatively charged site at Asp3 and a lone pair structure at His1. Although all five sites are required for complete binding to receptors with high affinity, substitutions at position 22 of 3-OCH3-4-OH-Phe and Lys12, Nle17, Val26, Thr28-VIP produced the best results, increasing potency by 4 times (IC50 = 4.4 nM vs. 15 nM) over VIP28 [55](52). Higher affinity may enhance tumor uptake and improve image quality. Again, our recent preliminary data in humans, obtained using Tc-99m-TP3654, a VIP analogue are consistent with this hypothesis [43, 68].

PACAP, a 38-amino acid peptide, isolated from bovine hypothalamus [69], was named PACAP because it stimulated the accumulation of intracellular and extracellular cAMP in monolayer cultures of rat anterior pituitary cells [70]. PACAP, a neurotransmitter and member of the VIP family, is 10 times more potent than VIP in stimulating adenylate cyclase in pituitary cells [70]. PACAP has three gene receptors, PACAP1, 2, and 3. Gottschall et al [69] isolated 27-amino acid PACAP (PACAP27) from bovine hypothalamus and concluded that PACAP38 and PACAP27 were equally active and derived from a single 176-amino acid precursor. PACAP27, like VIP, has an amidated C-terminus and histidine at the N-terminus. Nineteen of the 27 amino acids of PACAP27 are identical to VIP. It is for these reasons we chose to investigate a PACAP analogue TP3805 which also has higher IC50 value than VIP28.

### 1.4.2 Synthesis and Biological Evaluation of VIP and PACAP Analogues:

**Synthesis of N2(S-benzyl)2 Containing VIP:**

Vasoactive intestinal peptide (VIP) bound to a C-terminal diaminodithiol (N2S2) chelator was synthesized on a Wang resin using an ABI 341A peptide synthesizer (Applied Biosystems Inc.) [37] [30, 38]. The analogues were prepared, purified and characterized by American Peptide Company (Sunnyvale, CA) and named after their molecular weights as TP3805 and TP3939. A schematic presentation of TP3939 is given in Fig. 4. Although peptides have been conjugated with chelating agents such as DOTA (1, 4, 7, 10-tetraazadocdecane –N, N", N'", N"", -tetra acetic acid) it requires a prepared and pre-purified peptide to which DOTA is to be conjugated. The conjugated product then needs further purification and characterization. Preparation of our analogues is a one step process that provides efficiency, saves time and provides a N2S2 type of chelating moiety for strong chelation with Cu-64 or Tc-99m.

### 1.4.3 Preclinical Animal Studies:

Preclinical animal studies included i) TP3805 functional studies to assess its biochemical characteristics, ii) cell binding studies to examine its receptor specificity and (Kd) binding affinity, iii) receptor blocking studies to confirm its

receptor specificity, iv) tissue distribution studies in athymic nude mice bearing human PC tumors, v) imaging (PET/CT) spontaneously grown PC in transgenic TRAMP mice and vi) in vivo stability (30, 37, 38). As per the suggestion by CCRRC reviewers, the details of these data are eliminated from this revised application.

### 1.5 Toxicity Studies:

We plan to inject no more than 20 µg of a peptide to a standard 70 kg patient. We planned to inject i.v. to a group of three adult female rabbits, each 10 times, 50 times, and 100 times excess dose, adjusted to body weight (i.e. 2.86 µg/kg, 14.3 µg/kg, or 28.6 µg/kg). Preparations were made using decayed Cu-64 solution, as described previously. We initiated our work with three adult female, white New Zealand rabbits (age > 15 wks, body weight > 3 kg). The fourth rabbit served as a control. On day-1 of the experiment, each rabbit was weighed in a calibrated, electronic scale by placing a rabbit in a plastic box, which was placed on the scale and its weight tared. Animal body weights were read and recorded on an individual chart. All rabbit work described below was approved by the Institutional Animal Care and Use Committee and carried out with assistance from a trained technologist from the institutional laboratory animal services department. A staff veterinarian was available for consultation.

The animals (named A, B, and C) were then placed in a rabbit restraint box and two arterial blood samples, 3 mL each, were obtained from ear artery of each rabbit. One anti-coagulated sample was then given to the institutional hematology laboratory. The other was centrifuged and serum was collected. Part of the serum was retained in our laboratory for cAMP (cyclic Adenosine MonoPhosphate) assay, and the other portion was provided to the institutional clinical laboratory for determination of comprehensive metabolic panel. This procedure was followed on each rabbit. Each rabbit was then given 28.6 µg x body weight of peptide preparation in 500 µL 0.8% NaCl through a lateral ear vein and the syringe was flushed with another 500 µL of 0.9% NaCl. Animals were watched for any reaction for 10 minutes and another pair of 3 mL arterial blood samples was obtained through ear artery. Each rabbit was then watched twice daily, and on day 5, 10, and 17 they were weighed, weight recorded, and two venous blood samples from each rabbit were obtained for cAMP measurements and for blood and serum chemistry as described above. After drawing blood sample, on day 17, animals were sacrificed and samples of liver, spleen, kidney, lungs, and heart were removed. The tissues were then placed in 10% formalin; the tissues (5 x 3 treated rabbits plus 5 from a normal, untreated rabbit) were given to institutional histology research laboratory to obtain 5 µ thick, H+E stained histology slides. These slides were then examined by Dr. Peter McCue, Professor of Pathology at our (TJU) hospital.

cAMP assays were performed in triplicate on a total of 15 blood samples from the three experimental rabbits, along with the standards provided with a cAMP direct immunoassay kit obtained from Biovision (Mountain View, CA). A calibration curve was prepared and assays were performed in triplicate. Optical

density was measured at 450 nm. The readings were averaged for each serum sample and percent change in cAMP with respect to the sample obtained before peptide administration were calculated and tabulated.

### 1.5.1. Results of toxicology studies

#### i) Comprehensive metabolic panel

Results are tabulated in Table-2, in parts I and II. For all electrolytes, enzymes and protein measurements, each rabbit sample prior to injecting the peptide served as its own control. Results are presented as % difference for each rabbit at each time point and then as % average of the difference. The % difference averaged out < 25% except for bilirubin, which averaged 100%. We believe this is within the normal fluctuation range of 0.1 mg/dL to 1.2 mg/dL.

**Table-2, Part I: Comprehensive Metabolic Panel**

| Comprehensive Metabolic Panel Results | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Protein | % Diff. | Albumin | % Diff. | Calcium | % Diff. | Glucose | % Diff. | Creatinine | % Diff. | Total Bill | % Diff. | Alk Phos | % Diff. | AST (SGOT) | % Diff. |
| A1 (11/09) | 4.20 | 0.0 | 3.40 | 0.0 | 13.3 | 0.0 | 139.0 | 0.0 | 1.4 | 0.0 | 0.2 | 0.0 | 49.0 | 0.0 | 21.0 | 0.0 |
| A2 (11/09) | 4.50 | 7.1 | 3.50 | 2.9 | 13.1 | 1.5 | 172.0 | 23.7 | 1.3 | 7.1 | 0.2 | 0.0 | 50.0 | 2.0 | 17.0 | 19.0 |
| A3 (11/14) | 4.60 | 9.5 | 3.50 | 2.9 | 13.7 | 3.0 | 132.0 | 5.0 | 1.2 | 14.3 | 0.2 | 0.0 | 43.0 | 12.2 | 17.0 | 19.0 |
| A4 (11/19) | 4.20 | 0.0 | 3.30 | 2.9 | 13.3 | 0.0 | 137.0 | 1.4 | 1.3 | 7.1 | 0.2 | 0.0 | 41.0 | 16.3 | 14.0 | 33.3 |
| A5 (11/26) | 4.80 | 14.3 | 3.60 | 5.9 | 14.3 | 7.5 | 91.0 | 34.5 | 1.3 | 7.1 | 0.3 | 50.0 | 42.0 | 14.3 | 26.0 | 23.8 |
| % Diff Avg. | | 6.19 | | 2.94 | | 2.41 | | 12.9 | | 7.14 | | 10 | | 8.98 | | 19.05 |
| | | | | | | | | | | | | | | | | |
| B1 (11/09) | 4.40 | 0.0 | 3.40 | 0.0 | 13.7 | 0.0 | 146.0 | 0.0 | 1.1 | 0.0 | 0.1 | 0.0 | 72.0 | 0.0 | 21.0 | 0.0 |
| B2 (11/09) | 4.40 | 0.0 | 3.50 | 2.9 | 13.6 | 0.7 | 199.0 | 36.3 | 1.0 | 9.1 | 0.2 | 100.0 | 70.0 | 2.8 | 22.0 | 4.8 |
| B3 (11/14) | 4.40 | 0.0 | 3.50 | 2.9 | 13.2 | 3.6 | 142.0 | 2.7 | 0.9 | 18.2 | 0.1 | 0.0 | 67.0 | 6.9 | 21.0 | 0.0 |
| B4 (11/19) | 4.50 | 2.3 | 3.60 | 5.9 | 14 | 2.2 | 141.0 | 3.4 | 1.0 | 9.1 | 0.2 | 100.0 | 60.0 | 16.7 | 21.0 | 0.0 |
| B5 (11/26) | 4.60 | 4.5 | 3.60 | 5.9 | 14.1 | 2.9 | 149.0 | 2.1 | 0.9 | 18.2 | 0.2 | 100.0 | 54.0 | 25.0 | 17.0 | 19.0 |
| % Diff Avg. | | 1.36 | | 3.53 | | 1.90 | | 8.90 | | 10.91 | | 60 | | 10.28 | | 4.76 |
| | | | | | | | | | | | | | | | | |
| C1 (11/09) | 4.50 | 0.0 | 3.60 | 0.0 | 13.6 | 0.0 | 164.0 | 0.0 | 1.2 | 0.0 | 0.1 | 0.0 | 48.0 | 0.0 | 12.0 | 0.0 |
| C2 (11/09) | 4.80 | 6.7 | 3.70 | 2.8 | 13.2 | 2.9 | 201.0 | 22.6 | 1.2 | 0.0 | 0.3 | 200.0 | 50.0 | 8.7 | 12.0 | 0.0 |
| C3 (11/14) | 4.90 | 8.9 | 3.80 | 5.6 | 13.7 | 0.7 | 158.0 | 3.7 | 1.1 | 8.3 | 0.2 | 100.0 | 44.0 | 4.3 | 16.0 | 33.3 |
| C4 (11/19) | 4.60 | 2.2 | 3.60 | 0.0 | 13.7 | 0.7 | 162.0 | 1.2 | 1.2 | 0.0 | 0.2 | 100.0 | 40.0 | 13.0 | 15.0 | 25.0 |
| C5 (11/26) | 4.8 | 6.7 | 3.8 | 5.6 | 14.1 | 3.7 | 156 | 3.7 | 1.2 | 0.0 | 0.2 | 100.0 | 41 | 10.9 | 16 | 33.3 |
| % Diff Avg. | | 4.89 | | 2.78 | | 1.62 | | 6.22 | | 1.67 | | 100 | | 7.39 | | 18.33 |

**Table-2, Part II: Comprehensive Metabolic Panel**

| Comprehensive Metabolic Panel Results Part II | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ALT(SGPT) | % Diff. | Urea-N | % Diff. | Sodium | % Diff. | Potassium | % Diff. | Chloride | % Diff. | $CO_2$ | % Diff. | Anion Gap | % Diff. |
| A1 (11/09) | 28 | 0.0 | 24.0 | 0.0 | 141.0 | 0.0 | 4.3 | 0.0 | 106.0 | 0.0 | 22.0 | 0.0 | 13 | 0.0 |
| A2 (11/09) | 26 | 7.1 | 26.0 | 8.3 | 140 | 0.7 | 4 | 7.0 | 111 | 4.7 | 19 | 13.6 | 10 | 23.1 |
| A3 (11/14) | 27 | 3.6 | 25.0 | 4.2 | 142 | 0.7 | 4.3 | 0.0 | 112 | 5.7 | 21 | 4.5 | 9 | 30.8 |
| A4 (11/19) | 21 | 25.0 | 17.0 | 29.2 | 140 | 0.7 | 4 | 7.0 | 108 | 1.9 | 25 | 13.6 | 7 | 46.2 |
| A5 (11/26) | 29 | 3.6 | 25.0 | 4.2 | 143 | 1.4 | 4.2 | 2.3 | 105 | 0.9 | 22 | 0.0 | 16 | 23.1 |
| % Diff Avg. | | 7.86 | | 9.17 | | 0.71 | | 3.26 | | 2.64 | | 6.36 | | 24.6 |
| | | | | | | | | | | | | | | |
| B1 (11/09) | 57 | 0.0 | 20.0 | 0.0 | 141 | 0.0 | 4.4 | 0.0 | 108 | 0.0 | 25 | 0.0 | 8 | 0.0 |
| B2 (11/09) | 56 | 1.8 | 22.0 | 10.0 | 141 | 0.0 | 3.9 | 11.4 | 111 | 2.8 | 19 | 24.0 | 11 | 37.5 |
| B3 (11/14) | 55 | 3.5 | 20.0 | 0.0 | 140 | 0.7 | 4 | 9.1 | 110 | 1.9 | 21 | 16.0 | 9 | 12.5 |
| B4 (11/19) | 52 | 8.8 | 21.0 | 5.0 | 141 | 0.0 | 4.3 | 2.3 | 110 | 1.9 | 21 | 16.0 | 10 | 25.0 |
| B5 (11/26) | 62 | 8.8 | 19.0 | 5.0 | 140 | 0.7 | 4.2 | 4.5 | 198 | 83.3 | 23 | 8.0 | 8 | 0.0 |
| % Diff Avg. | | 4.56 | | 4.00 | | 0.28 | | 5.45 | | 17.96 | | 12.8 | | 15 |
| | | | | | | | | | | | | | | |
| C1 (11/09) | 29 | 0.0 | 21.0 | 0.0 | 139 | 0.0 | 4.1 | 0.0 | 110 | 0.0 | 21 | 0.0 | 8 | 0.0 |
| C2 (11/09) | 30 | 3.4 | 22.0 | 4.8 | 139 | 0.0 | 3.9 | 4.9 | 111 | 0.9 | 17 | 19.0 | 11 | 37.5 |
| C3 (11/14) | 28 | 3.4 | 24.0 | 14.3 | 141 | 1.4 | 3.8 | 7.3 | 111 | 0.9 | 21 | 0.0 | 9 | 12.5 |
| C4 (11/19) | 27 | 6.9 | 21.0 | 0.0 | 140 | 0.7 | 3.7 | 9.8 | 108 | 1.8 | 24 | 14.3 | 8 | 0.0 |
| C5 (11/26) | 31 | 6.9 | 24 | 14.3 | 139 | 0.0 | 4.1 | 0.0 | 108 | 1.8 | 22 | 4.8 | 9 | 12.5 |
| % Diff Avg. | | 4.14 | | 6.67 | | 0.43 | | 4.39 | | 1.09 | | 7.62 | | 12.5 |

| Abbreviations |
|---|
| Total Bili = Total Bilirubin |
| Alk Phos = Alkaline Phosphatase |
| AST(SGOT) = Aspartate aminotransferase |
| ALT(SPGT) = Alanaine aminotransferase |
| Urea-N = Urea-Nitrogen |

**ii) Complete blood count and differential**

Complete blood and blood differential analysis was performed on each rabbit blood sample obtained prior to peptide injection (control) and then at 10 min, 5d, 10d, and 17d post injection. Data are summarized in Table-3, parts I and II. The data are presented as % difference with respect to the control sample for the hematological parameters in each blood sample obtained after administration of the diagnostic agent. In none of the data points (except last reading for WBC in animal A) was the difference greater than 6% (well below 10%). These data too are consistent with the lack of evidence that the agent had induced any pharmacologic effect.

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## Table-3, Part I: Comprehensive Blood Panel

| Comprehensive Blood Panel Results | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WBC | % Diff | RBC | % Diff | HGB | % Diff | HCT | % Diff | MCV | % Diff | MCH | % Diff |
| A1 (11/09) | 5.30 | 0.0 | 5.46 | 0.0 | 12.1 | 0.0 | 36.9 | 0.0 | 68 | 0.0 | 22.2 | 0.0 |
| A2 (11/09) | 5.60 | 5.7 | 5.73 | 4.9 | 12.8 | 5.8 | 38.8 | 5.1 | 68 | 0.0 | 22.3 | 0.5 |
| A3 (11/14) | 6.64 | 25.3 | 5.31 | 2.7 | 11.9 | 1.7 | 37.2 | 0.8 | 70 | 3.1 | 22.4 | 0.9 |
| A4 (11/19) | 4.80 | 9.4 | 5.10 | 6.6 | 11.4 | 5.8 | 36.3 | 1.6 | 71 | 4.4 | 22.4 | 0.9 |
| A5 (11/26) | 7.40 | 39.6 | 5.91 | 8.2 | 13.4 | 10.7 | 41.7 | 13.0 | 71 | 4.4 | 22.7 | 2.3 |
| % Diff Avg. | | 16.00 | | 4.51 | | 4.79 | | 4.12 | | 2.38 | | 0.90 |
| | | | | | | | | | | | | |
| B1 (11/09) | 6.90 | 0.00 | 5.81 | 0.00 | 12.6 | 0.00 | 38.4 | 0.00 | 66 | 0.00 | 21.7 | 0.00 |
| B2 (11/09) | 6.50 | 5.80 | 5.78 | 0.52 | 12.4 | 1.59 | 38.3 | 0.26 | 66 | 0.00 | 21.5 | 0.92 |
| B3 (11/14) | 6.20 | 10.14 | 5.07 | 12.74 | 10.9 | 13.49 | 34.4 | 10.42 | 68 | 3.03 | 21.5 | 0.92 |
| B4 (11/19) | 6.10 | 11.59 | 5.51 | 5.16 | 12.1 | 3.97 | 38.0 | 1.04 | 69 | 4.55 | 22.0 | 1.38 |
| B5 (11/26) | 6.90 | 0.00 | 5.72 | 1.55 | 12.6 | 0.00 | 39.9 | 3.91 | 70 | 6.06 | 22.0 | 1.38 |
| % Diff Avg. | | 5.51 | | 3.99 | | 3.81 | | 3.13 | | 2.73 | | 0.92 |
| | | | | | | | | | | | | |
| C1 (11/09) | 5.50 | 0.00 | 6.02 | 0.00 | 12.5 | 0.00 | 38.5 | 0.00 | 64 | 0.00 | 20.8 | 0.00 |
| C2 (11/09) | 5.10 | 7.27 | 6.34 | 5.32 | 13.2 | 5.60 | 40.6 | 5.45 | 64 | 0.00 | 20.8 | 0.00 |
| C3 (11/14) | 5.60 | 1.82 | 5.90 | 1.99 | 12.3 | 1.60 | 38.4 | 0.26 | 65 | 1.56 | 20.8 | 0.00 |
| C4 (11/19) | 4.90 | 10.91 | 5.65 | 6.15 | 11.8 | 5.60 | 37.3 | 3.12 | 66 | 3.13 | 20.9 | 0.48 |
| C5 (11/26) | 4.9 | 10.91 | 6.14 | 1.99 | 12.8 | 2.40 | 40.6 | 5.45 | 66 | 3.13 | 20.8 | 0.00 |
| % Diff Avg. | | 6.18 | | 3.09 | | 3.04 | | 2.86 | | 1.56 | | 0.10 |

**Table-3, Part II: Comprehensive Blood Panel**

| Comprehensive Blood Panel Results Part II | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MCHC | % Diff | RDW | % Diff | PLT | % Diff | MPV | % Diff |
| A1 (11/09) | 32.8 | 0.00 | 13.9 | 0.00 | 231 | 0.00 | 7.8 | 0.00 |
| A2 (11/09) | 33.0 | 0.61 | 14.0 | 0.72 | 223 | 3.46 | 7.7 | 1.28 |
| A3 (11/14) | 32.0 | 2.44 | 15.4 | 10.79 | 236 | 2.16 | 7.6 | 2.56 |
| A4 (11/19) | 31.4 | 4.27 | 15.0 | 7.91 | 236 | 2.16 | 7.4 | 5.13 |
| A5 (11/26) | 32.1 | 2.13 | 14.6 | 5.04 | 251 | 8.66 | 7.5 | 3.85 |
| % Diff Avg. | | 1.89 | | 4.89 | | 3.29 | | 2.56 |
| | | | | | | | | |
| B1 (11/09) | 32.8 | 0.00 | 13.2 | 0.00 | 284 | 0.00 | 8.1 | 0.00 |
| B2 (11/09) | 32.4 | 1.22 | 13.1 | 0.76 | 271 | 4.58 | 8.0 | 1.23 |
| B3 (11/14) | 31.7 | 3.35 | 14.4 | 9.09 | 273 | 3.87 | 7.9 | 2.47 |
| B4 (11/19) | 31.8 | 3.05 | 14.5 | 9.85 | 275 | 3.17 | 7.8 | 3.70 |
| B5 (11/26) | 31.6 | 3.66 | 14.2 | 7.58 | 275 | 3.17 | 7.9 | 2.47 |
| % Diff Avg. | | 2.26 | | 5.45 | | 2.96 | | 1.98 |
| | | | | | | | | |
| C1 (11/09) | 32.5 | 0.00 | 13.4 | 0.00 | 257 | 0.00 | 7.2 | 0.00 |
| C2 (11/09) | 32.5 | 0.00 | 13.5 | 0.75 | 261 | 1.56 | 7.3 | 1.39 |
| C3 (11/14) | 32.0 | 1.54 | 14.0 | 4.48 | 240 | 6.61 | 7.2 | 0.00 |
| C4 (11/19) | 31.6 | 2.77 | 13.5 | 0.75 | 235 | 8.56 | 7.5 | 4.17 |
| C5 (11/26) | 31.5 | 3.08 | 13.3 | 0.75 | 244 | 5.06 | 7.2 | 0.00 |
| % Diff Avg. | | 1.48 | | 1.34 | | 4.36 | | 1.11 |

### ii) Body Weight

The animal body weights are summarized in Table-4. No animal suffered any body weight loss, suggesting that the peptide administration induced no adverse events leading to reduced dietary intake, altering animal body chemistry, or physiology that would adversely influence body weight. In fact, there was only an increase in the body weight, which is an expected normal phenomenon indicating normal health in all three animals.

**Table-4: Rabbit Body Weights. Peptide injected: 28.5 ug/Kg. Total Peptide Injected: Rabbit A: 99.47 ug Rabbit B: 88.92 ug Rabbit C: 93.20 ug**

| Day | Date | Rabbit A | Rabbit B | Rabbit C |
|---|---|---|---|---|
| Day 0(injection day) | | | | |
| Before injection | 11-08-2007 | 3.49kg | 3.12kg | 3.27kg |
| 10 min after injection | 11-08-2007 | Same | Same | Same |
| Day 5 | 11-13-2007 | 3.53kg | 3.14kg | 3.24kg |
| Day 10 | 11-19-2007 | 3.60kg | 3.15kg | 3.30kg |
| Day 17(sacrificed) | 11-26-2007 | 3.66kg | 3.27kg | 3.43kg |

### iii) Cyclic Adenosine Monophosphate

The data on determination of serum cAMP (pmol/mL) in all three rabbits are given in Table-5. Data strongly show that in none of the rabbits, at any time during the 17 day experimental period, was there any statistically significant change (p = 0.34 or greater) in cAMP. The normal serum cAMP range in rabbits is 12.76-20.93 pmol/mL. The cAMP values in rabbits B and C were well within this range. In rabbit A however, the control serum cAMP value was 21.095. At the 10 min time point, this value was 21.78 and at day 10 it was 22.22. These values are not statistically, significantly different within themselves. These data too indicate that the cAMP levels were not altered by the administration of the peptide.

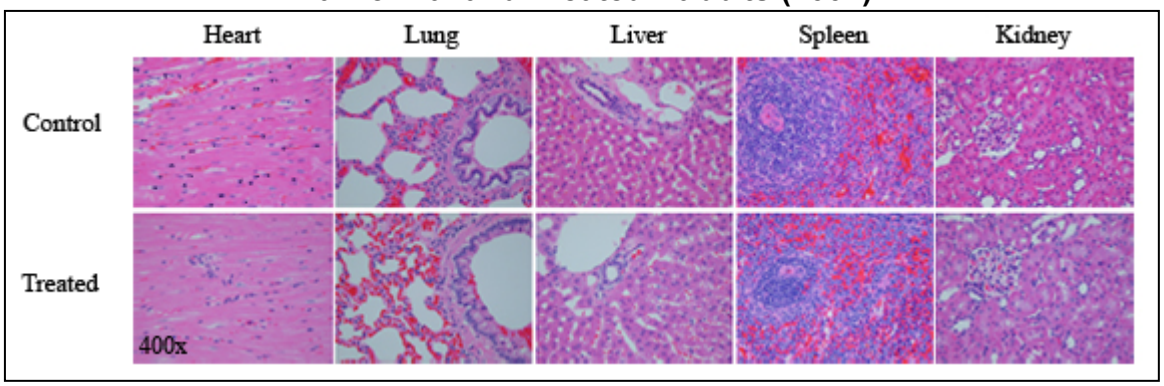
**Table-5: Rabbit Serum cAMP Levels**

| cAMP Concentration (pmol/mL) | | | | |
|---|---|---|---|---|
| **Date** | **Rabbit** | **Rabbit** | **Rabbit** | **Average** |
| **Day 0 (Injection Day) Before Injection** | 21.095 | 16.505 | 12.95 | 16.85±4.085 |
| **Day 0 (Injection Day) 10 min After Injection** | 21.78 | 16.42 | 16.59 | 18.26±3.05 (p = 0.66) |
| **Day 5** | 19.36 | 16.67 | 15.03 | 17.02±2.19 (p = 0.915) |
| **Day 10** | 22.22 | 16.67 | 12.95 | 17.28±4.67 (p = 0.91) |
| **Day 17 (sacrifice)** | 12.26 | 16.07 | 13.82 | 14.05±1.92 (p = 0.34) |

**iv) Histology**

The animal histology data are presented in Table-6 (signed by Dr. McCue, Professor of Pathology) and in Fig. 5 as stated by Professor McCue, no specific pathologic changes in organs examined were induced at any time in any of the animals.

**Figure 5: Pathologic Representation of Tissue from a Normal and Treated Rabbits (400x)**



## Table-6: Pathologic Changes in Normal and Treated Rabbits

Thomas Jefferson University Hospital

Jefferson Health System

Surgical Pathology

January 7, 2008

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Jefferson Hospital for Neuroscience
Ford Road Campus
Methodist Hospital Nursing Center

Re: Experimental Tissues

Dear Dr. Thakur:

I have reviewed the normal tissue controls and the injected animals. I found no histopathologic change between the experimental groups.

Light Microscopic Results (Table 6)

| Specimen | Heart | Lung | Liver | Spleen | Kidney | | |
|---|---|---|---|---|---|---|---|
| N | NSPC* | NSPC | NSPC | NSPC | NSPC | | |
| A | NSPC | NSPC | NSPC | NSPC | NSPC | | |
| B | NSPC | NSPC | NSPC | NSPC | NSPC | | |
| C | NSPC | NSPC | NSPC | NSPC | NSPC | | |

* No Specific Pathologic Changes

Sincerely,

Peter A. McCue, MD
Professor of Pathology
Thomas Jefferson Health System
10th & Walnut
Philadelphia, PA 19107

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### v) Conclusion

These data in all three rabbits for 17 days after the administration of the highest (x100) peptide concentration dose did not show any pharmacologic effect in any parameter studied. We therefore concluded that the studies in lower concentration (x10 and x50) doses would be unnecessary, and we could not justify sacrificing so many otherwise normal rabbits.

## 1.6 Radiation Dosimetric Calculations

The mouse tissue distribution data obtained at 4hr and 24hr post injection was converted into % ID/organ and normalized for a kg body weight. All organs were weighed and average weight was used. Blood weight was estimated at 7% of the body weight, intestine at 40% body weight (32) and muscle at 9.3% body weight (33). Dosimetric calculations were performed using OLINDA/EXM 1.0 program obtained from Vanderbilt University. Data, the effective dose equivalent, is presented in Table-7 in mSv/mBq and rem/mCi.

**Table-7: Estimated Absorbed Radiation Dose for Cu-64-Peptide**

| | Estimated Radiation Dose | |
|---|---|---|
| Target Organ | mSv/MBq | rem/mCi |
| | | |
| Adrenals | 1.81E-02 | 6.71E-02 |
| Brain | 3.10E-04 | 1.15E-03 |
| Breasts | 4.83E-03 | 1.79E-02 |
| Gallbladder Wall | 2.71E-02 | 1.00E-01 |
| LLI Wall | 8.77E-03 | 3.25E-02 |
| Small intestine | 2.09E-01 | 7.72E-01 |
| Stomach Wall | 1.05E-02 | 3.87E-02 |
| ULI Wall | 2.17E-02 | 8.01E-02 |
| Heart Wall | 1.43E-01 | 5.30E-01 |
| Kidneys | 2.73E-01 | 1.01E+00 |
| Liver | 2.47E-01 | 9.14E-01 |
| Lungs | 4.08E-02 | 1.51E-01 |
| Muscle | 1.17E-02 | 4.32E-02 |
| Ovaries | 1.27E-02 | 4.70E-02 |
| Pancreas | 1.66E-02 | 6.14E-02 |
| Red Marrow | 6.73E-03 | 2.49E-02 |
| Osteogenic Cells | 4.63E-03 | 1.71E-02 |
| Skin | 2.71E-03 | 1.00E-02 |
| Spleen | 3.20E-03 | 3.45E-01 |
| Thymus | 7.74E-03 | 2.86E-02 |
| Thyroid | 1.81E-03 | 6.71E-03 |
| Urinary Bladder Wall | 4.31E-03 | 1.59E-02 |
| Uterus | 1.16E-02 | 4.28E-02 |
| Total Body | 1.76E-02 | 6.53E-02 |

For whole body imaging, (Group-A, please see section 1) we shall enroll 6 patients. In order to determine optimal image quality, we shall inject 2 patients with 3 mCi (±10%), 2 patients with 3.5 mCi (±10%) and 2 with 4 mCi (±10%). Image quality will be determined by a physician. Imaging time shall be approximately 45 mins.

Patients in group B will undergo PEM imaging using only the dose as determined from above. However, this dose shall not be more than 4±10% mCi. It is estimated that the dose received by kidneys, (total) blood, gall bladder wall, lungs, pancreas, uterus, and osteogenic cells will be high among all organs. However, the overall absorbed radiation dose is less than that from the commonly used diagnostic dose of 5-10 mCi of Ga-67 (Table-8) (34).

**Table-8: Radiation Dosimetry for Ga-67**

| | Estimated Radiation Dose (mGy/MBq) | |
|---|---|---|
| ORGAN | mGy/MBq | rad/mCi |
| | | |
| Adrenals | 1.3E-01 | 4.7E-01 |
| Brain | 5.4E-02 | 2.0E-01 |
| Breasts | 4.6E-02 | 1.7E-01 |
| Gallbladder Wall | 8.3E-02 | 3.1E-01 |
| LLI Wall | 2.6E-01 | 9.7E-01 |
| Small Intestine | 8.6E-02 | 3.2E-01 |
| Stomach | 6.9E-02 | 2.6E-01 |
| ULI Wall | 1.5E-01 | 5.4E-01 |
| Heart Wall | 6.7E-02 | 2.5E-01 |
| Kidneys | 1.1E-01 | 4.2E-01 |
| Liver | 1.1E-01 | 4.2E-01 |
| Lungs | 6.1E-02 | 2.3E-01 |
| Muscle | 5.9E-02 | 2.2E-01 |
| Ovaries | 8.7E-02 | 3.2E-01 |
| Pancreas | 7.9E-02 | 2.9E-01 |
| Red Marrow | 1.2E-01 | 4.6E-01 |
| Bone Surfaces | 3.2E-01 | 1.2E+00 |
| Skin | 4.4E-02 | 1.6E-01 |
| Spleen | 1.4E-01 | 5.2E-01 |
| Testes | 5.5E-02 | 2.0E-01 |
| Thymus | 5.9E-02 | 2.2E-01 |
| Thyroid | 6.0E-02 | 2.2E-01 |
| Urinary Bladder Wall | 9.0E-02 | 3.3E-01 |
| Uterus | 7.9E-02 | 2.9E-01 |
| | | |
| Effective Dose Equivalent | 1.1E-01 mSv/MBq | 4.1E-01 rem/mCi |

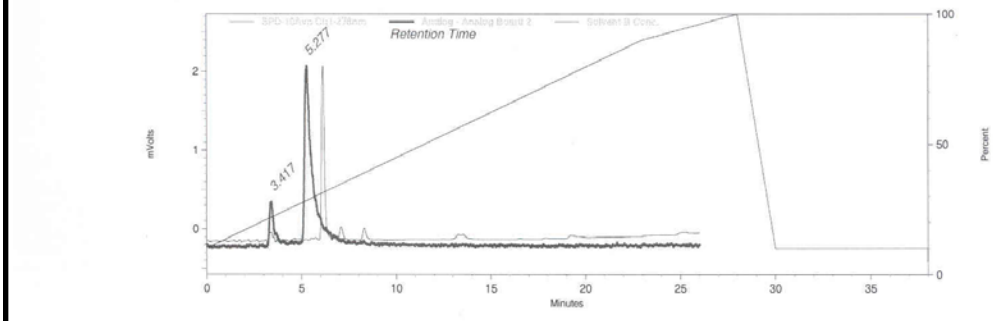
Please note that we expect the lung absorbed dose to be much smaller. This is because the mice lungs from which this human absorbed dose was derived had micrometastases in their lungs, secondary to the primary breast cancer. In normal lung mice, the uptake is only 25% of that seen here. Therefore, in the studies with human lungs, free of cancer or cancer metastases, we anticipate much less absorbed radiation dose than presented here.

## 1.7 Chemistry preparations and controls (CMC)

### 1.7.1 Preparation

The preparation will consist of 20 µg of the peptide in 4 µL of sterile 0.1M acetate buffer pH = 4.6, to which will be added 400 µL of 0.2 M glycine buffer, pH = 9, 100 µg of $SnCl2{\cdot}2H_2O$ in 20 µL 0.05M HCl, and 1000 µg of glucoheptonate in 20 µL sterile water, and up to 10 mCi of Cu-64 in 1-2 µL 0.1M HCl. The mixture will be heated for 90 min. at 50°C. (Please note: Since we have increased the dose to be administered, we have increased the quantity of radioactivity to be added to the vial. This has changed the incubation time and temperature. This change has no influence on the pharmacokinetics or the biological activity of the preparation).

HPLC analysis will be performed. A typical HPLC profiles given in Fig. 6 shows 97.4% radiochemical purity.




Analysis: Radiation Detector

Analog - Analog
Board 2 Results

| Pk # | Time | Height | Area | Area Percent |
|---|---|---|---|---|
| 1 | 3.471 | 469 | 342 | 2.612 |
| 2 | 5.277 | 2172 | 12749 | 97.388 |
| Totals | | 2641 | 13091 | 100.000 |

Fig. 6: Typical Cu-64-TP3805 HPLC Analysis

The mixture will then be diluted with 500 µL of 0.9% sterile NaCl solution, filtered through 0.22 µ filter. 4±10% mCi of this solution will be drawn into a one mL, sterile syringe for injection.

## 1.8 Clinical Data to Date:

**PET Imaging of Human Breast Cancer Using Cu-64-TP3805:**

Human BC also overexpresses VPAC1 receptor (Fig. 3). Fig. 2.a, Fig. 2.b and 2.c show that Cu-64 TP3805can detects not only the primary BC (six out of six malignant lesions in four patients studies so far) but also the sentential lymph nodes. These data provide the evidence that the Cu-64-TP3805 analogue data in mouse model, can be successfully translated to accurately image human tumors and provide a confidence that this PC imaging investigation is worthwhile.

In the USA and abroad, prostate cancer (PC) takes thousands of lives every year. Yet its early and accurate detection continues to be challenging. At the onset of PC, VPAC1, which belongs to the superfamily of G-protein coupled receptors, expresses in high density on PC cells, but not on benign or normal cells. Our extensive preclinical findings, and preliminary data in humans, have shown that $^{64}$Cu-TP3805 has a high affinity for VPAC1, is stable in vivo, and has the ability to image breast tumors in humans and spontaneously grown PC in TRAMP mice. The goal was to determine the ability of $^{64}$Cu-TP3805 to image PC in men and to evaluate the findings with pathologic examinations.

**Methods**: 25 men scheduled for radical prostatectomy and who signed IRB approved consent form were PET imaged preoperatively. Standardized Uptake Values (SUVs) were determined, malignant lesions (SUV > 1.0) were counted, and data compared with histologic findings. In addition, de-paraffinized whole mount pathology slides (n=68) from 6 VPAC1 PET imaged patients, 3 benign prostatic hyperplasia (BPH) patients (n=9), one malignant lymph node (n=3), and one benign lymph node (n=3) were incubated with $^{64}$Cu-TP3805, washed with PBS, dried and subjected to digital autoradiography (DAR). Slides were then H&E stained, read by a pathologist, and DAR images were compared with foci delineated as PC, benign, cyst, or prostatic intraepithelial neoplasia (PIN).

**Results**: In 25 patients, 212 lesions had SUVs > 1.0 as compared to 127 lesions identified by histology. While the status of the remaining 85 PET lesions remains to be determined, in 68 histological slides from 6 PET imaged patients, DAR identified 105/107 PC foci (missed 2 due to artifact), 19/19 high grade PIN (HGPIN), 2/2 ejaculatory ducts (ED) and 5/5 urethra verumontanum (UV). Additionally, DAR found 9 PC lesions not previously identified by histology. The positive and the benign lymph nodes were correctly identified by DAR, and for the 3/3 BPH patients without PC, and 5/5 cysts, DAR was negative.

**Conclusion**: $^{64}$Cu-TP3805, a VPAC1 receptor specific biomolecule delineates PC lesions in vivo and ex vivo, provides normal images for benign masses, and is worthy of further studies in patients.

In this investigation, we will use the same preparation, Cu-64-TP3805 that has been prepared and used in the previoius human subjects with 100% success. Although no preparation will either be compounded or used out of the Jefferson campus, we will use a “kit” that will be ready to be heated following the

addition of 440 µL of sterile H2O and then Cu-64 activity. These kits will contain all chemicals in sterile, 10 mL glass vials. Reagents will be added to the vial, snap frozen in dry ice, lyophilized and the vials will be sealed in N2 atmosphere. They will be stored at 4°C and examined for Cu-64 labeling and shelf life stability.

Such preparations will be convenient and assure consistency of reagents. Such kits prepared previously in our laboratory were stable for more than two years. We expect similar stability, but will be used only after careful examination of their reproducible and stable preparations, as examined by HPLC. There will be only one patient studied at a time. The syringe containing the Cu-64-TP3805 will be clearly labeled with patient's name. "Cu-64-TP3805" name, the amount of radioactivity, and the time at which the dose was dispensed.

Please note that under this eIND 101550 and with all institutional approvals, the agent was administered to nineteen females with known BC. All malignant lesions including involved lymph nodes were unequivocally delineated (100%). There were no adverse events except three patients sensed flushing which resolved without any medication within a few minutes. Therefore, we will not change this preparation (Cu-64-TP3805). A preprint (J Nucl Med July 2013) of this investigation, reference [79].

### 1.9 Dose Rationale and Risk/Benefits

During the nineteen BC patient studies as per the suggestion by the FDA, a dose escalation study was performed using 3 mCi±10% (n=2), 3.5 mCi±10% (n=2), and 4 mCi±10% (n=2). From these studies, it was concluded that the image quality was much better with a 4 mCi±10% Cu-64-TP3805 dose while the radiation dose (liver target organ) to the liver and all other organs were well within the dose delivered by routine diagnostic nuclear medicine procedures. Based on this, FDA approved the use of 4 mCi±10% dose to the next thirteen patients. In this PC PET study therefore, we will use 4 mCi±10% dose which is also approved by the FDA (eIND 101550) for this PC PET study. We will strictly adhere to this dose.

## 2.0 STUDY OBJECTIVES

### 2.1 Primary Objective:

The primary objective of this study is to assess the ability of Cu-64-TP3805 to detect Prostate cancer in patients with persistently elevated PSA.

## 3.0 STUDY DESIGN

### 3.1 General Design

This is a single institution, open-label pilot phase I feasibility diagnostic imaging study.

### 3.2 Primary Study Endpoints

The primary endpoint of study will be to determine the ability of Cu-64-TP3805 to detect PC foci within the prostate gland in patients with persistialy elevated PSA as compared with tumor maps derived by histology of biopsy specimens obtained guided biopsy.

### 3.3 Primary Safety Endpoints

As stated previously in section 1.9, we will use 4 mCi±10% Cu-64-TP3805 dose given with FDA approval to fifteen of the nineteen BC patients. The peptide quality (20 µg) will also be kept unchanged.

There was no toxicity in this entire study of the nineteen subjects except three subjects sensed flushing which resolved within minutes without any medication. It is reasonable therefore to anticipate that in this PC study too, there will be no adverse events (AE) due to dose administered. Nevertheless, a physician (either Dr. Intenzo or Dr. Kim) will be available during and after the injection. An up to date first aid box will be available. The Research Coordinator will call each subject 24 hrs later and make note of any AE patients may have experienced.

## 4.0 SUBJECT SELECTION AND WITHDRAWAL

### 4.1 Inclusion Criteria

Subjects must meet all of the following criteria to be enrolled in this study:

1. Male aged 21 years or older.
2. Ability to provide signed informed consent and willingness to comply with protocol requirements.
3. Have persistently elevated PSA.
4. Scheduled to have biopsy at least 7days post Cu-64.
5. Agree to use an acceptable form of birth control for a period of 7 days after the Cu-64-TP3805 injection.

### 4.2 Exclusion Criteria

Subjects must not meet any of the following criteria to be enrolled in this study:

1. Participating would significantly delay the scheduled standard of care therapy.
2. Administered a radioisotope within 10 physical half-lives prior to study drug injection.
3. Have any medical condition or other circumstances that, in the opinion of the investigator, would significantly decrease obtaining reliable data, achieving study objectives or completing the study.

### 4.3 Gender/Minority/Pediatric Inclusion for Research

This study will include men of any race or ethnicity who meet eligibility criteria. As PC is a disease of middle aged and older men, no women or pediatric patients will be included.

### 4.4 Subject Recruitment and Screening

A total of 20 patients with persistently elevated PSA, presented at Urology Associates Office of TJU, and are scheduled for a biopsy, will be enrolled. Drs. E. Trabulsi, C. Lallas, and L. Gomella shall determine the patient eligibility, and inform the PI, Dr. Mathew Thakur, and the research coordinator, Nancy Pedano. Each patient shall sign an IRB approved consent form. Each patient shall be administered IV with 4 mCi ± 10% Cu-64-TP3805.

### 4.5 Early Withdrawal of Subjects

#### 4.5.1 When and How to Withdraw Subjects

Patients who withdraw consent or who do not complete the investigational imaging or who do not have radical prostatectomy will be withdrawn from study and will not be included in the analysis.

#### 4.5.2 Data Collection and Follow-up for Withdrawn Subjects

Withdrawn subjects will be removed from the analysis and no follow up will be undertaken.

## 5.0 STUDY IMAGING AGENT

### 5.1 Description

In this investigation, we will use the same preparation, Cu-64-TP3805 that has been prepared and used in nineteen previous human BC subjects with 100% success. Although no preparation will either be compounded or used out of the Jefferson campus, we will use a “kit” that will be ready to be heated following the addition of 440 µL of sterile H2O and then Cu-64 activity. These kits will contain all chemicals in sterile, 10 mL glass vials. Reagents will be added to the vial, snap frozen in dry ice, lyophilized and the vials will be sealed in N2 atmosphere. They will be stored at 4°C and examined for Cu-64 labeling and shelf life stability. Such preparations will be convenient and assure consistency of reagents. Such kits prepared previously in our laboratory were stable for more than two years. We expect similar stability, but will be used only after careful examination of their reproducible and stable preparations, as examined by HPLC. There will be only one patient studied at a time. The syringe containing the Cu-64-TP3805 will be clearly labeled with patient’s name. All preparations were sterile. However, sterility will be examined on each dose.

### 5.2 Treatment Regimen

A protocol assistant from the Urology Department will assist with accrual and enrollment, and a clinical coordinator from the Radiology Department will guide the patient through the imaging procedure. IRB approved informed consent

will be obtained by one of the investigators. The patients will be asked to read the IRB-approved consent form and encouraged to ask any questions they deem necessary. Four ±10% mCi of Cu-64-TP3805, prepared as described here in will be injected through an indwelling intravenous line and the lines will be flushed with 5 ml sterile 0.9% saline. Patient vital signs (heart rate, respiratory rate, blood pressure) will be monitored before administration, at 15 minute post injection and then at the end of the scan. Patients will be scanned at 90 minutes with whole body PET/CT. Imaging time will be approximately one hour. Patients will be contacted over the phone 24 hours later and asked for any unlikely adverse events. Any AE shall be recorded and notified immediately to IRB, FDA and to CCRRC.

Whole body PET/CT imaging will be acquired by a trained and experienced technologist at Jefferson Center City Imaging facility located within the TJU campus. Images will be read either by Dr. Intenzo or Dr. Kim. Data will be recorded. Care will be taken of patient privacy.

### 5.3 Risks

Based on the previous nineteen recipients of Cu-64-TP3805, we do not anticipate any adverse events after the i.v. administration of peptide analog, the patients will remain in the clinic for at least two hours and observed either by the technologist or by the research coordinator. Dr. Kim and Dr. Intenzo will be available should their expertise be required. A first aid box equipped appropriately will be at hand all the time.

Patient vital signs (heart rate, respiratory rate, blood pressure) will be monitored before administration, at 15 minute post injection and then at the end of the scan. Patients will be scanned at 90 minutes with whole body PET/CT The nursing staff will also watch for any flushing or redness. Each reading will be recorded.

Twenty-four hours later, we will telephone each patient to ask for any adverse events the patient may experience. These will be recorded.

### 5.4 Method for Assigning Subjects to Treatment Groups

Not applicable.

### 5.5 Preparation and Administration of Study Imaging Agent

The Cu-64 peptide analog will be prepared using the methods described previously. Sterile reagents and glassware will be used. The dose, no more than five mL in volume, will be dispensed. Sterility tests will be performed and recorded. To maintain the procedure uniformity, we will prepare kits and use them for instant Cu-64 labeling. HPLC analysis will also be performed for determination of radiochemical purity and data recorded.

Four ±10% mCi of Cu-64-TP3805, prepared as described here in will be injected through an indwelling intravenous line and the lines will be flushed with 5 ml sterile 0.9% saline.

### 5.6 Subject Compliance Monitoring

Not applicable.

### 5.7 Prior and Concomitant Therapy

Not applicable.

## 6.0 STUDY PROCEDURES

### 6.1 Study Visit Schedule (see appendix 15.1)

**Screening**

Potential subjects identified by the urologic oncologists (Drs. Trabulsi, Lallas and Gomella) who have persistently elevated PSA, 4 or greater and scheduled for MRI fusion/TRUS . The clinical coordinator will screen the patient for eligibility as per the inclusion and exclusion criteria. Eligible subjects will review and sign the informed consent. The coordinator will schedule the Cu-64-TP3805 PET imaging study at least one week but not more than 3 weeks before their scheduled MRI Fusion/TURS biopsy

**Visit 1**

The clinical coordinator will meet the subject and guide them through the imaging process. They will monitor the patient before, during and after the imaging study, as previously described.

**Follow-up**

The clinical coordinator will follow up with the subjects by phone the day after the study imaging procedure to ensure that there were no adverse reactions. The coordinators will be available as needed for the time period until their scheduled biopsy procedure for questions or concerns.

## 7.0 STATISTICAL PLAN

### 7.1 Sample Size Determination

**7.2** We will study 20 patients with persistently elevated PSA for a Cu-64 PET imaging in whom biopsy are ordered. These patients shall be PET imaged with our Cu-64 peptide probe first and then a week or so later the image guided biopsy shall be performed. The image guided biopsy is the standard of care procedure. The objective is to validate the PET imaging results with the histology of biopsy results. Generally, approximately 60% of the patients with elevated PSA have prostate cancer. We expect 80% accuracy in identifying 20 subjects as having histologically determined cancer/absence of cancer. The 95% confidence interval of this level of accuracy for this sample size is + or – 17.5%. Thus we expect to find an accuracy level between 62.5% to 97.5%. Please note this is a pilot study, not designed to be an efficacy determination trial. **Statistical Methods**

### 7.3 Subject Population(s) for Analysis

20 subjects completing the investigational imaging and undergoing biopsy and PET imaged with 4 mCi ± 10% Cu-64-TP3805.

### 7.4 Replacement of Participants

Participants who are consented but are not dosed will be replaced

## 8.0 SAFETY AND ADVERSE EVENTS

### 8.1 Definitions

**Adverse Event**

An AE is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

**Serious Adverse Event**

Adverse events are classified as serious or non-serious.

**A serious adverse event is any AE that is:**

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as **non-serious adverse events**.

**Adverse Event Reporting Period**

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 24 hours following the study imaging procedure.

**Preexisting Condition**

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

**General Physical Examination Findings**

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

**Post-study Adverse Event**

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

## 8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded

The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

### 8.3 Data and Safety Monitoring Plan

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the compliance and implementation of the KCC data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events by both the assigned Medical Monitor and the KCC DSMC.

#### 8.3.1 Medical Monitoring and AE/SAE Reporting

A Medical Monitor is assigned to this study at the Thomas Jefferson University. This is a physician/pharmacist who is not directly involved in the trial, and is not currently collaborating with the sponsor/investigator on any other trial. The role of the Medical Monitor is to review all reportable AEs/SAEs (in real-time) including grading, toxicity assignments, non-reportable AEs (quarterly), protocol violations/deviations, as well as all other safety data and activity data observed in the ongoing clinical trial occurring at Thomas Jefferson University. The Medical Monitor may recommend reporting of adverse events and relevant safety data, and may also recommend suspension or termination of the study to the DSMC and TJU IRB.

Every KCC investigator initiated protocol includes requirements for reporting of adverse events based on CTC 4.0. All events are reported to the IRB and Medical Monitor using a password protected web-site. In addition all unexpected and serious adverse events (SAEs) are reported to the TJU IRB and to the Food and Drug Administration (FDA) if applicable. The investigator is required to submit all unexpected and serious adverse events to the TJU IRB and the Medical Monitor within the timeframes outlined in the below table. All AE/SAEs will be reported to the DSMC at the quarterly DSMC review meetings; however, if the Medical Monitor determines corrective action is necessary, an "ad hoc" DSMC meeting will be called. **Fatal adverse events related to treatment which are unexpected must be reported within 24 hours to the TJU IRB and the DSMC. Fatalities not related to the study drug/device must be reported within 5 days.** A summary of the reporting requirements for KCC investigator initiated Phase I and Phase II studies are presented below.

| | Grade 1 | Grade 2 | Grade 2 | Grade 3 | | Grade 3 | | Grades 4 and 5 |
|---|---|---|---|---|---|---|---|---|
| | Unexpected and Expected | Unexpected | Expected | Unexpected with Hospitalization | Unexpected without Hospitalization | Expected with Hospitalization | Expected without Hospitalization | Unexpected and Expected |
| Unrelated Unlikely | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | Reviewed at Quarterly DSMC Meeting and IRB | 5 Working Days | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | 5 Working Days | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | Phase 1 - 48 Hours (Death: 24 Hours)<br><br>Phase 2 - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Annual Review | | | | | 5 Working Days |
| Possible Probable Definite | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | 5 working days | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | 48 Hours (Death: 24 Hours) | Phase 1 - 48 Hours Phase 2 - 5 Working Days | 48 Hours (Death: 24 Hours) | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | Phase 1 and Phase 2 - 48 Hours (Death: 24 Hours |

**NOTE: This table is based on the NCI AE/SAE reporting Guidelines and the TJU IRB Policy and Procedures. Please follow the individual protocol AE/SAE reporting guidelines if more stringent reporting procedures are specified.

**Safety Reporting Requirements for IND Holders**

In accordance with 21 CFR 212.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements:

**Expedited IND Safety Reports:**

7 Calendar-Day Telephone or Fax Report:

The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of Cu-64-TP3805. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Such reports are to be telephoned or faxed to the FDA within 7 calendar days and insert funding sponsor within 24 hours of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND

15 Calendar-Day Written Report:

The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the use of Cu-64-TP3805. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, insert funding sponsor, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these

reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g. summary letter).

**IND Annual Reports**

In accordance with the regulation 21 CFR § 312.32, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report. All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to insert funding sponsor. Copies of such reports should be mailed to: NIH, Program Director Dr. H. Baker

### 8.3.2 Data and Safety Monitoring Committee

- Data and Safety Monitoring Committee (DSMC) is the Data and Safety Monitoring Board (DSMB) for the KCC. The DSMC is a multidisciplinary committee charged with overseeing the monitoring of safety of participants in clinical trials, and the conduct, progress, validity, and integrity of the data for all clinical trials at the Thomas Jefferson University KCC. The committee meets quarterly to review the progress and safety of all active research protocols that are not monitored by another safety and data monitoring committee or board.
- The DSMC meets quarterly. Additional DSMC meetings are scheduled based on the nature and number of trials being monitored over a specified time period. The DSMC meets (by conference call) within 24 hours following the notification of an unexpected adverse event felt to be related to the study drug.
- Prior to each DSMC meeting, each board member, is provided a printout of all reported AEs and SAEs occurring during the reporting period for this clinical trial. The principal investigator provides a detailed and comprehensive narrative assessment of current adverse events to date, indicating their possible significance and whether these toxicities have affected the conduct of the trial. DSMC members are provided with the principal investigator's assessment, a written report summarizing adverse events, safety data, and activity data observed during the specified time period described in each protocol, as well as recommendations from the Medical Monitor. A review of outcome results (response, toxicity and adverse events) and factors external to the study (such as scientific or therapeutic developments) is discussed, and the Committee votes on the status of each study.
- A summary of the board's action is sent to each investigator, the CCRRC and Thomas Jefferson University IRBs. The DSMC actions may include recommendations/requirements that will lead to improved patient safety and/or efficacy, significant benefits or risks that have developed, or other changes determined to be necessary. The DSMC may also take note of

slow accrual or lack of scientific progress, and refer such issues to the CCRRC. The DSMC provides the investigator with the rationale for any decision made.

## 9.0 DATA HANDLING AND RECORD KEEPING

### 9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

### 9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

### 9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it.

All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

### 9.4 Records Retention

This is a FDA regulated study. It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

## 10.0 STUDY MONITORING, AUDITING, AND INSPECTING

### 10.1 Study Monitoring Plan

The investigator will allocate adequate time for monitoring activities. The Investigator will also ensure that the medical monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

**KCC Investigator Initiated Phase I Studies**

Phase I studies require continuous monitoring by the PI of the study with quarterly safety and monitoring reports submitted to the CRMO and the DSMC. Each protocol is assigned to a medical monitor (a physician or other member of the DSMC who has expertise in the therapeutic area of the protocol and is not directly involved in this trial). The medical monitor reviews all adverse events (in addition to unexpected adverse events), safety data and activity data observed in the ongoing clinical trial at each new dose level, prior to dose escalation.

The PI provides a report to the DSMC of all AE/SAEs, safety and toxicity data, and any corrective action that occurred on a quarterly basis. The medical monitor also provides a summary of their review. The summary of all discussions of adverse events are submitted to the DSMC, and these reports are reviewed during the DSMC meetings that take place quarterly. Patients are only identified by initials, and no other personal health information (PHI) is included in the reports.

The medical monitor may recommend reporting adverse events and relevant safety data not previously reported, and may recommend suspension or termination of the trial based on their review of AE/SAE data observed throughout the life of a clinical trial. In such circumstances, and "ad hoc" DSMC meeting will be called to discuss corrective action with the PI. If for any reason the PI of the trial disagrees with the conclusions of the Medical Monitor or DSMC,

the issue will be referred to the Associate Director of Clinical Investigations, who will be responsible for dispute resolution.

The summary of all discussions of adverse events are included in the KCC investigator's reports to the TJU IRBs as part of its annual progress report. The DSMC and the TJU IRBs may, based on the monitor's recommendation suspend or terminate the trial. The quarterly safety and monitoring reports include a statement as to whether this data has invoked any stopping criteria in the clinical protocol.

## 10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the funding sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

### 10.2.1 Independent External and Internal Audits

In addition to review by the DSMC, all studies initiated by KCC investigators are audited by an independent auditor once they have achieved 10% of target accrual. However, a study can be audited at any time based on recommendations by the IRB, DSMC, CCRRC and/or the Director of Clinical Investigations, KCC. Studies are re-audited once they have achieved 50% of target accrual. Special audits may be recommended by the IRB, DSMC or CCRRC based on prior findings, allegations of scientific misconduct and where significant irregularities are found through quality control procedures. Any irregularities identified as part of this process would result in a full audit of that study.

In addition to the audits at 10 and 50%, the CRMO randomly audits at least 10 percent of all patients entered into therapeutic KCC trials and other trials as necessary, on at least a bi-annual basis, to verify that there is a signed and dated patient consent form, the patient has met the eligibility criteria, and that SAEs are documented and reported to the TJU IRB.

All audit reports are submitted to the DSMC for review and action (when appropriate). A copy of this report and recommended DSMC action is sent to the CCRRC and TJU IRB. The committee regards the scientific review process as dynamic and constructive rather than punitive. The review process is designed to assist Principal Investigators in ensuring the safety of study subjects and the adequacy and accuracy of any data generated. The TJU IRB may, based on the DSMC and auditor's recommendation, suspend or terminate the trial.

## 11.0 ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

All subjects for this study will be provided a consent form that is compliant with local and federal regulations, describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

## 12.0 STUDY FINANCES

### 12.1 Funding Source

This study is financed through a grant from the US National Institute of Health, through the National Cancer Institute.

### 12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Jefferson University Investigators will follow the TJU Conflicts of Interest Policy for Employees (107.03).

### 12.3 Subject Stipends or Payments

To compensate for their travel, parking, meals and a partial loss of wages, each patient will be paid at the completion of the study, one hundred fifty dollars.

## 13.0 PUBLICATION PLAN

All data shall be published in a peered reviewed open access journal. Data will also be presented at national and international gatherings. An appropriate acknowledgment to NIH will be made.

## 14.0 REFERENCES

This is the bibliography section for any information cited in the protocol. It should be organized as any standard bibliography.

## References


1. American_Cancer_Society (2009) Cancer Facts and Figures. 2009; Available from: http://www.cancer.org/downloads/STT/2008CAFFfinalsecured.pdf.
2. Bronson, J.G., Imaging Prostate Cancer: Before, During and After Treatment. Decisions in Imaging Economics, in Journal of Imaging Technology Magazine 2001. p. 1-19.
3. Loeb S, R.K., Antenor JA, et al. (2006) Baseline prostate-specific antigen compared with medical prostate-specific antigen for age group as predictor of prostate cancer risk I men younger than 60 years old. Urology 67(2):316-320.
4. Catalona, W.J., Smith, D.S., and Ornstein, D.K. (1997) Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination. Enhancement of specificity with free PSA measurements. JAMA 277(18):1452-5.
5. Lelievre, V., Pineau, N., and Waschek, J.A. (2003) Biological Significance of PACAP and PACAP receptors in human tumors: From cell line to cancers. , in Pituitary adenylate cyclase-activating polypeptide. .
6. Reubi, J.C. (1995) In vitro identification of vasoactive intestinal peptide receptors in human tumors: implications for tumor imaging. J Nucl Med 36(10):1846-53.
7. Reubi, J.C., Laderach, U., Waser, B., Gebbers, J.O., Robberecht, P., and Laissue, J.A. (2000) Vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor subtypes in human tumors and their tissues of origin. Cancer Res 60(11):3105-12.
8. Reubi, J.C. (2000) In vitro evaluation of VIP/VPAC receptors in Healthy and diseased human tissues, in Proceedings of the 4th Int. Sym. On VIP PACAP, Glucagon related peptides, J.F.a.S. Said, Editor. Annals of NY Acad. Sc. , 1-25.
9. Thakur, M.L., Marcus, C.S., Saeed, S., Pallela, V., Minami, C., Diggles, L., Pham, H.L., Ahdoot, R., Kalinowski, E.A., and Moody, T. (2000) Imaging tumors in humans with Tc-99m-VIP. Ann N Y Acad Sci 921:37-44.
10. Pallela, V.R., Thakur, M.L., Chakder, S., and Rattan, S. (1999) 99mTc-labeled vasoactive intestinal peptide receptor agonist: functional studies. J Nucl Med 40(2):352-60.
11. Kolan HR Pallela V, a.T.M. (1997) Tc-99m labeled Vasoactive Intestinal Peptide (VIP): Preparation and preliminary evaluation. . J Labelled Compounds and Radiopharmaceuticals 40:455-457.
12. Thakur, M.L., Marcus, C.S., Saeed, S., Pallela, V., Minami, C., Diggles, L., Le Pham, H., Ahdoot, R., and Kalinowski, E.A. (2000) 99mTc-labeled vasoactive intestinal peptide analog for rapid localization of tumors in humans. J Nucl Med 41(1):107-10.

13. Rao, P.S., Thakur, M.L., Pallela, V., Patti, R., Reddy, K., Li, H., Sharma, S., Pham, H.L., Diggles, L., Minami, C., and Marcus, C.S. (2001) 99mTc labeled VIP analog: evaluation for imaging colorectal cancer. Nucl Med Biol 28(4):445-50.
14. Terris, M. (2000) Extended Field Prostate Biopsies: Too Much of a Good thing? Urology 55:457-460.
15. Gottlieb, R., Mooney, C, Mushlin, A et al (1996) The Prostate:Decreasing Cost-Effectiveness of Biopsy with Advancing Age. Investigative Radiology 31(2):84-90.
16. Bietendorf, J. (2004) FDG PET Reimbursement. J. Nucl. Med. Tech. 32(1):33-38
17. Wingo, P.A., Cardinez, C.J., Landis, S.H., Greenlee, R.T., Ries, L.A., Anderson, R.N., and Thun, M.J. (2003) Long-term trends in cancer mortality in the United States, 1930-1998. Cancer 97(12 Suppl):3133-275.
18. Jadvar, H. (2009) Molecular Imaging of Prostate Cancer: A Concise Synopsis. Molecular Imaging 8(2):56-64.
19. Kurhanewicz J, S.M.N.S., et al (2002) Combined magnetic resonance imaging and spectroscopic imaging approach to molecular imaging of prostate cancer. J Magn Reson Imaging. 16(4):451-463.
20. Song SK, Q.Z., Garabedian EM, et al (2002) Improved magnetic resonance imaging detection of prostate cancer in a transgenic mouse model. Cancer Res. 62(5):1555-1558.
21. Alonzi R, P.A., Allen C (2007) Dynamic contrast enhanced MRI in prostate cancer. Eur J Radiol 63:335-350.
22. Jackson ASN, R.S., Sobhaib SA, et al (2009) Dynamic contrast-enhanced MRI for prostate cancer localization. The British Journal of Radiology 82:148-156.
23. Fricke, S.T., Rodriguez, O., Vanmeter, J., Dettin, L.E., Casimiro, M., Chien, C.D., Newell, T., Johnson, K., Ileva, L., Ojeifo, J., Johnson, M.D., and Albanese, C. (2006) In vivo magnetic resonance volumetric and spectroscopic analysis of mouse prostate cancer models. Prostate 66(7):708-17.
24. Westphalen AC, K.J., Cunha RM, et al. (2009) T2-Weighted endorectal magnetic resonance imaging of prostate cancer after external beam radiation therapy. IInt Braz J Urol. 35(2):171-180.
25. McMahon CJ, B.B., Lenkinski RE, et al. (2009) Dynamic contrast-enhanced MR imaging in the evaluation of patients with prostate cancer. magn Reson Imaging Clin N Am. 17(2):363-383.
26. Scheffel, U. and Pomper, M.G. (2004) PET imaging of GRP receptor expression in prostate cancer. J Nucl Med 45(8):1277-8.

27. Chen X, P.R., Hou Y, et al (2004) microPET and Autoradiographic Imaging of GRP Receptor Expression with 64Cu-DOTA-[Lys3] Bombesin in Human Prostate Adenocarinoma Xenografts. J Nucl Med. (45):1390-1397.
28. Pillarsetty NV, P.B., Larson SM (2009) 2-18F-Fluoropropionic Acid as a PET Imaging Agent for Prostate Cancer. J Nucl Med 50:1709-1714.
29. Farsad M, S.R., Franceschelli A, et al (2008) Positron-emission tomography in imaging and staging prostate cancer. Cancer Biomarkers 4(4-5):277-284.
30. Zhang, K., Aruva, M.R., Shanthly, N., Cardi, C.A., Rattan, S., Patel, C., Kim, C., McCue, P.A., Wickstrom, E., and Thakur, M.L. (2008) PET Imaging of VPAC1 Expression in Experimental and Spontaneous Prostate Cancer. Journal of Nuclear Medicine 49(1):112-121.
31. Peng F, L.X., Janisse J et al (2006) PET of human prostate cancer xenografts in mice with increased uptake of 64CuCl2. J Nucl Med 47(10):1649-1652.
32. Virgolini, I., Raderer, M., Kurtaran, A., Angelberger, P., Banyai, S., Yang, Q., Li, S., Banyai, M., Pidlich, J., Niederle, B., Scheithauer, W., and Valent, P. (1994) Vasoactive intestinal peptide-receptor imaging for the localization of intestinal adenocarcinomas and endocrine tumors. N Engl J Med 331(17):1116-21.
33. Gespach, C., Bawab, W., de Cremoux, P., and Calvo, F. (1988) Pharmacology, molecular identification and functional characteristics of vasoactive intestinal peptide receptors in human breast cancer cells. Cancer Res 48(18):5079-5083.
34. Leylon F, P.J., Jakolew S, et al (1998) VIP1 receptors are present in breast cancer biopsy specimens. Cancer Research 38:111.
35. Moody TW, L.J., Unsworth E, et al (1998) (Arg15, Arg 21) VIP: A VIP agonist for localizing breast cancer tumors. Peptides 19:585-592.
36. Thakur, M.L. (2009) Genomic Biomarkers for Molecular Imaging: Predicting the Future. Seminars in Nuclear Medicine 39:236-246.
37. Thakur ML, A.M., Garippa R, et al (2004) PET Imaging of Oncogene Overexpression Using Cu-64-VIP analog: Comparison with Tc-99m-VIP analog. . J Nucl Med. 45:1381-1389.
38. Zhang K, A.M., Shanthly N, et al. (2007) Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating peptide (PACAP) receptor specific peptide analogues for PET imaging of breast cancer; In vitro/in vivo evaluation. Regulatory Peptides 144:91-100.
39. Jagoda EM, A.L., Seidei J, et al (2002) Comparison of an 18F labeled derivative of vasoactive intestinal peptide and 2-deoxy-2-[18F] fluoro-D-glucose in nude mice bearing breast cancer xenografts. Mol Imaging Biol 4(5):369-379.

40. Wienhard K, S.M., Casey ME, et al (2002) The ECAT HRRT: Performance and First Clinical Application of the New High Resolution Research Tomograph. IEEE Transactions on Nuclear Science 49(1).
41. Meltzer CC; Leal JP, M.H., et al (1990) Correction of PET Data for Partial Volume Effects in Human Cerebral Cortex by MR Imaging. Journal of Computer Assisted Tomography 14:561-570.
42. Pallela VR, T.M., Chakder, et al. (1999) Tc-99m labeled vasoactive intestinal peptide receptor agonist: functional studies. . J Nucl Med. 40:352-360.
43. Thakur ML, M.C., Saeed S, et al. (2000) 99m Tc-labeled Vasoactive Intestinal Peptide analog for rapid localization of tumors in humans. J Nucl Med. 41:107-110.
44. Zhang K, A.M., Shanthly N, et al. (2008) Pet imaging of VPAC1 expression in experimental and spontaneous prostate cancer. . J Nucl Med. 49:112-121.
45. Reubi JC, L.U., Waser B, et al (2000) Vasoactive Intestinal Peptide/Pituitary Adenylate Cyclase Activating Peptide receptor subtypes in human tumors and their tissues of origin. Cancer Res 69:1305-1312.
46. Said SI, M.V. (1970) Polypeptide with braod biological activity: Isolation from the small intestine. Science 69:1217-1218.
47. Chakder S, R.S.T.e.v.i.p.m.i.r.f.t.a.o.t.v.i.p.r.F.a.b.s.J.P.a.E.T. (1993) The entire vasoactive intestinal polypeptide molecule is required for the activation of the vasoactive intestinal polypeptide receptor: Functional and binding studies. J Pharm and Expt Therapeutics 266:392-399.
48. Blum AM, M.R., Cook GA, et al (1992) Murine mucosal T cells have VIP receptors functionally distinct from those on intestinal epithelial cells. J Neuroimmunol 39:101-108.
49. Couvineau A, L.M. (1985) The human vasoactive intestinal peptide receptor: Molecule identification by covalent cross-linking in colonic epithelium. J Clin Endocrinol Metab 61:50-55.
50. TW, M. (1995) Peptides and growth factors in non-small cell lung cancer. Peptides 17:545-555.
51. Paul S, S.S. (1987) Characterization of receptors for vasoactive intestinal peptide solubilized from the lung. J Biol Chem 262:158-162.
52. Sreedharan SP, R.A., Peterson KE, et al (1991) Cloning and expression of the human vasoactive intestinal peptide receptor. Proc Natl Acad Sci USA. 88:4986-4990.
53. el Battari A, M.J., Luis J, et al (1988.) Solubilization of the active vasoactive intestinal peptide receptor from human colonic adenocarinoma cells. J Biol Chem 263:17685-17689.

54. Reubi JC, W.B., Laderach U, et al (1997) Pituitary adenylate cyclase activating polypeptide (PACAP) and PACAP II receptors in human tumors. In vitro binding of DTPA-linked PACAP analogs. Eur J Nucl Med 24:1058.
55. Bolin, D.R., Cottrell, J., Garippa, R., Michalewsky, J., Rinaldi, N., Simko, B., and O'Donnell, M. (1995) Structure-activity studies on the vasoactive intestinal peptide pharmacophore. 1. Analogs of tyrosine. Int J Pept Protein Res 46(3-4):279-89.
56. Lewis JS, L.R., Dehadashfi F, et al (2008) An Imaging comparison with Cu-64-ATSM and Cu-60-ATSM in cancer of the Uterine Cervix. J. Nucl. Med 49:1177-1182.
57. Zia F, F.M., Bitar K, et al (1995) Pituitary adenylate cyclase activating peptide receptors regulate the growth of non-small cell lung cancer cells. Cancer Res 5:4886-4891.
58. Zia, H., Hida, T., Jakowlew, S., Birrer, M., Gozes, Y., Reubi, J.C., Fridkin, M., Gozes, I., and Moody, T.W. (1996) Breast cancer growth is inhibited by vasoactive intestinal peptide (VIP) hybrid, a synthetic VIP receptor antagonist. Cancer Res 56(15):3486-9.
59. Zia H, L.J., Loecho T, et al (1997) PACAP receptors are present on breast cancer cell lines. Am Assoc Cancer Res 38:117.
60. Le Meuth V, F.N., Bawab W, et al (1991) Characterization of binding sites for VIP-related peptides and activation of adenylate cyclase in developing pancreas. Amer J of Physiology 260:G265-74.
61. Basille M, G.B., Fournier A, et al (1994) Ontogeny of pituitary adenylate cyclase activating polypeptide (PACAP) receptors in the rat cerebellum: a quantitative autoradiographic study. Brain Research 82:1-2.
62. Vertrongen P, C.I., Darro F, et al (1996) VIP and pituitary adenylate cyclase activating polypeptide (PACAP) have an antiproliferative effect on the T98G human glioblastoma cell line through interaction with VIP2. Neuropeptides 30:491-496.
63. Olianas MC, E.M., Lampis G, et al (1996) Presence of pituitary adenylate cyclase activating polypeptide receptors in Y-79 human retinoblastoma cells. J Neurochem 67:1292-1300.
64. Lema-Kisoka, R., Hayez, N., Langer, I., Robberecht, P., Sariban, E., and Delporte, C. (2001) Characterization of functional VIP/PACAP receptors in the human erythroleukemic HEL cell line. Peptides 22(12):2155-62.
65. Lelievre V, B.-G.L., Meunier AC, et al (1996) Switches in the expression and function of PACAP and VIP receptors during phenotypic interconversion in human neuroblastoma cells. Neuropeptides 30:313-322.
66. Parkman HP, P.A., Ryan JP (1998) PACAP and VIP inhibit pyloric muscle through VIP/PACAP preferring receptors. Regulatory Peptides 71:185-190.

67. Bolin, D.R., Cottrell, J., Garippa, R., Rinaldi, N., Senda, R., Simko, B., and O'Donnell, M. (1996) Comparison of cyclic and linear analogs of vasoactive intestinal peptide. Drug Des Discov 13(3-4):107-14.
68. Thakur ML;Marcus CS Saeed S, e.a. (2000) Imaging tumors in humans with Tc-99m-VIP, in VIP, PACAP, Glucagon, and Related Peptides. Ann N Y Acad Sci.
69. Gottschall PE, T.I., Miyata A, et al (1990) Characterization and distribution of binding sites for the hypothalamic peptide, pituitary adenylate cyclase activating polypeptide. Endocrinology 127:272-277.
70. Miyata A, A.A., Dahl RR, et al (1989) Isolation of a novel 38-residue-hypothalamine polypeptide which stimulates adenylate cyclase in pituitary cells. Biochem Biophys Res Commun 164:567-574.
71. Thakur, M.L., Devadhas, D., Zhang, K., Pestell, R.G., Wang, C., McCue, P., and Wickstrom, E. (2010) Imaging spontaneous MMTVneu transgenic murine mammary tumors: Targeting metabolic activity versus genetic products. Journal of Nuclear Medicine 51(1):106-111.
72. Bostwick, D., Murphy, G (1995) High grade prostatic intraepithelial neoplasia: the most likely precursor of prostate cancer. Cancer 75:1823-36.
73. Thompson IM, L.M., Redman MW, et al (2007) Finasteride decreases the risk of prostatic intraepithelial neoplasia. J Urol 178:107-9.
74. Anderson, C.J., Dehdashti, F., Cutler, P.D., Schwarz, S.W., Laforest, R., Bass, L.A., Lewis, J.S., and McCarthy, D.W. (2001) 64Cu-TETA-octreotide as a PET imaging agent for patients with neuroendocrine tumors. J Nucl Med 42(2):213-21.
75. Smith, S.V. (2004) Molecular imaging with copper-64. J Inorg Biochem 98(11):1874-901.
76. Boswell, C.A., Sun, X., Niu, W., Weisman, G.R., Wong, E.H., Rheingold, A.L., and Anderson, C.J. (2004) Comparative in vivo stability of copper-64-labeled cross-bridged and conventional tetraazamacrocyclic complexes. J Med Chem 47(6):1465-74.
77. Schultz S, R.C., Mawrin C, et al (2004) Immunocytochemical Identification of VPAC1, VPAC2, and PAC1 Receptors in Normal and Neoplastic Human Tissues with Subtype-Specific Antibodies. American Association for Cancer Research 10:8235-8242.
78. Zheng, Y.Y. and Saluja, S. (1996) Gallium and Indium Complexes of Bis(amino thiol) N2S2) Ligands. Inorganic Chemistry. 35:6656-6666.
79. Thakur, M.L. Kaijun, Z., Berger, A, et al. VPAC1 receptors for imaging breast cancer: a feasibility study. (J Nucl Med, 54: No. 7 to be published 2013).
80. Wise AM, Stamey TA, McNeal JE, et al. (2002) Morphologic and clinical significance of multifocal PCs in radical prostatectomy specimens. Urology. 60:264–69.

## 15.0 APPENDICES

Include any attachments for this study (e.g. study schedule/visit chart from procedures section, Pill Diaries to be used, recruitment materials if applicable, AE Logs from section 5.2, Eligibility Checklist, Drug Reconciliation Form, etc.)

### 15.1 Visit Chart:

Subject with persistently high PSA seeing a Urologic Oncologist at Jefferson (Drs. Trabulsi, Lallas or Gomella) and is scheduled for MRI fusion/trans rectal ultrasound (TRUS) prostate biopsy .

⇓

**Screening Visit:**

- Clinical coordinator reviews chart and interviews patient
- Clinical coordinator confirms subject eligibility
- Subject signs informed consent

⇓

**Imaging Visit:**

- Clinical coordinator meets patient in Radiology
- Vitals signs are monitored before and after imaging procedure
- Intravenous catheter inserted and study agent (Cu-64-TP3805) infused
- PET imaging procedure completed
- Follow up call 24 hours later to ensure absence of adverse event

⇓

**MRI fusion/TURS Date:**

- At least one week but not more than 3 weeks later
- Biopsy complete and pathology results received
- Subject completion of protocol

## 15.2 Case Report forms:

Thomas Jefferson University

**Cu-64-TP3805**

**PET Imaging Date:_____**
**MRI fusion/TURS Biopsy Date:_____**

**Study #_____**

**Surgeon: _____**

**Patient Identification:**

**Patient Initials: _____** **Medical Record #: _____**

**Body Weight: _____** **Age: _____**

**Ethnicity: ☐ Caucasian ☐ African American**

**☐ Asian ☐ Latino**

**Inclusion Criteria:**

**☐ Intermediate/High Risk Disease (must check at least one box):**

**PSA : _____ ☐ PSA ≥10 ng/DL**

**DRE: _____ ☐ T2b, T2C, T3 or T4**

**Signature: _____**

Thomas Jefferson University

**StudyCu-64-TP3805 PET Scan results**
**Date: _____**

**Patient INT"L_____**

**Study #_____**

**Interpreting Radiologist: _____**

**Prostate Sextant Visible Foci:**

☐ **L Base SUV: _____** ☐ **R Base SUV: _____**

**Size: ___ x ___ x ___ mm** **Size: ___ x ___ x ___ mm**

☐ **L Mid SUV: _____** ☐ **R Mid SUV: _____**

**Size: ___ x ___ x ___ mm** **Size: ___ x ___ x ___ mm**

☐ **L Apex SUV: _____** ☐ **R Apex SUV: _____**

**Size: ___ x ___ x ___ mm** **Size: ___ x ___ x ___ mm**

**Signature: _____**