Imaging urothelial bladder cancer: A VPAC PET targeted approach

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Introduction: *Accurate staging of urothelial bladder cancer (UBC) with imaging, which guides effective bladder cancer treatment, remains challenging. This investigation is to validate a hypothesis that targeting Vasoactive intestinal and pituitary adenylate cyclase activating peptide (VPAC) receptors using ^{64}Cu-TP3805 can PET image UBC efficiently.*

Materials and methods: *Nineteen patients (44-84 years of age) scheduled for radical cystectomy, underwent VPAC positron emission tomography (PET) imaging prior to surgery. Sixteen had completed neoadjuvant chemotherapy prior to imaging. All 19 received ^{64}Cu-TP3805 (148 % ± 10% MBq) intravenously, and were imaged 60 to 90 minutes later. Standard uptake value (SUV)max for malignant lesions and SUVmean for normal tissues were determined and mean ±SEM recorded. Following radical cystoprostatectomy, pelvic lymphadenectomy and urinary diversion imaging, results were compared with final surgical pathology.*

Results: *^{64}Cu-TP3805 had no adverse events, negligible urinary excretion and rapid blood clearance. UBC PET images for residual disease were true positive in 11 patients and true negative in four. Of remaining 4, one had false positive and 3 had false negative scans, equating to 79% sensitivity (95%, CI 49%-95%), 80% specificity (95%, CI 28%-100%), 92% positive predictive value (95%, CI 62%-100%) and 57% negative predictive value (95%, CI 18%-90%).*

Conclusions: *These first in man results, in a group, heavily pretreated with neoadjuvant chemotherapy, indicate that VPAC PET imaging can identify UBC effeiciently and suggest, that VPAC PET can diagnose UBC in a treatment naïve cohort for accurate staging, guide biopsy and treatment in patients with suspected metastasis and determine response to therapy. Further investigation of this molecular imaging approach is warranted.*

Key Words: ^{64}Cu-TP3805, bladder cancer, computed tomography, positron emission tomography, radioisotope diagnostic technique

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Introduction

In 2020, in the USA more than 80,000 new cases of urothelial bladder cancer (UBC) were diagnosed and more than 17,000 UBC deaths were accounted.[1] UBC is a heterogeneous disease, more prevalent in men (60%) than in women (40%). Although at the time of initial diagnosis, approximately 80% of UBC are non-muscle invasive, the remaining are invasive and/or metastatic. UBC metastasizes with high propensity to lymph nodes (69%), bone (47%), lungs (37%), liver (26%)[2,3] and involves the prostate in 30.9% of the patients.[4] UBC treatment is stage dependent. The prognosis for locally advanced UBC (transmural disease pT3+ or node positive) and metastatic disease is poor, with a subgroup analysis of the 5 year survival after radical cystectomy and urinary diversion for node negative patients of 67%, declining to 31% for node positive patients.[5] Accurate identification of these patients would change their management, favoring upfront systemic therapy. Local consolidation with extirpative surgery or radiotherapy would be reserved for those either with good radiographic response, or treated for palliation only. The ability of conventional crossectional imaging to accurately predict stage and nodal status is poor and inconsistent for UBC. In a meta-analysis of 35 published series, a reported pooled sensitivity for the detection of lymph node metastasis for CT was 40%; 60% for magnetic resonance imaging (MRI); and 56% for FDG PET.[6] Standard FDG PET also suffers from urinary excretion of the FDG tracer, which imposes challenges for accuracy of local staging, and therefore is not currently recommended for UBC workup.[7,8]

Furthermore, because the bladder is a multi-layer organ, accurate local staging remains challenging, leaving as many as 30% to 50% of patients under-staged at the time of cystectomy.[9] There is a clear unmet need for accurate staging of newly diagnosed locally advanced and metastatic UBC, which would guide treatment.

Carbon-11-choline has minimal urinary excretion and is incorporated into tumor cells by conversion into ^{11}C-phosphatidyl choline which is a significant component of cell membranes, for which ^{11}C-choline has been investigated for PET imaging of UBC.[7,8] The sensitivity and specificity of ^{11}C-choline for imaging nodal metastasis has been reported to be higher than those with contrast enhanced CT.[8,10] However, due to the short (20 min.) half-life of Carbon-11, its use is limited only to a few medical centers in the country, located in the close vicinity of a cyclotron which is required to produce it. A recent article further discusses the need for improved agents to image UBC .[11]

Our investigational approach to PET image UBC stems from the fact that VPAC (combined for vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide), G-protein receptors, are expressed in high density on UBC cells.[12] VPAC receptors are also expressed in high density on many other tumor cells.[13-18] A 27 amino acid peptide, PACAP, binds with high affinity to VPAC receptors.[12] In order to target VPAC receptors to PET image oncologic diseases, our laboratory has designed, synthesized and evaluated a PACAP analogue, (^{64}Cu-TP3805) which has a high affinity ($3.1x10^{-9}$M) for VPAC, no immunogenicity and the ability to image spontaneous breast and prostate in transgenic mice.[13-16] Furthermore translational investigations have successfully demonstrated PET imaging of breast and prostate cancer in humans with high sensitivity.[17-19] Digital autoradiographic studies of a bladder cancer microarray, using ^{64}Cu-TP3805, have demonstrated higher uptake of ^{64}Cu- TP3805 for aggressive bladder cancer than that for low grade bladder cancer.[19] Additionally, in preclinical and human studies in other malignancies, we have observed[17,18] that ^{64}Cu-TP3805 has negligible urinary excretion. With these favorable attributes, we hypothesized that targeting VPAC surface receptors with ^{64}Cu-TP3805 in vivo, will allow us to PET image UBC in humans, an area of unmet need.

Materials and methods

Informed consent

Each patient enrolled, signed a Institutional Review Board (IRB) approved informed consent form and received a copy of it.

Compliance and protocol approvals

The study was approved by institutional Protocol Review Committee (PRC), Radioactive Drug Research Committee (RDRC), and Institutional Review Board (IRB #16G.500). The Institutional Clinical Research Organization (CRO), provided the protocol oversight.

Patient inclusion

Ninteen Patients (M, 44-84 yrs.) diagnosed with invasive UBC, scheduled for radical cystoprostatectomy, pelvic lymphadenectomy and urinary diversion, were enrolled. Out of these, 16 patients had completed their standard of care, neoadjuvant chemotherapy regimen. Three patients, who were not eligible for standard of care neoadjuvant chemotherapy, were taken straight to surgery.

Copper-64-TP3805 administration, PET imaging and SUVmax/SUVmean determinations

Following informed consent, subjects were injected through a cubital vein in one arm, with 148% ± 10% MBq, ^{64}Cu-TP3805 in 3 mL of sterile 0.9% NaCl. Vital signs were monitored at 15 minutes prior to and then immediately after, and at 15 min., 30 min., 60 min., and 2 hrs. post-injection. Using an indwelling heparinized i.v. catheter in the other arm, 3 mL blood samples were drawn at 1, 10, 30, 60, and 120 min. post injection. Voided urine was collected at the end of whole body PET/CT imaging which commenced between 60-90 min. post-injection of ^{64}Cu-TP3805. Siemens Bigraph-6 PET/CT scanner was used for imaging from head to knee with 4 min. bed time. Images were read independently by two board certified nuclear medicine physicians who were aware of the patient general inclusion criteria but not of the detailed individual patient history, at the time of reading. The physicians also determined maximum standard uptake value (SUVmax) for each detectable lesion and SUVmean for the highest metabolic activity in each organ including left and right lungs and kidneys by using one square cm regions of interest. The MIM software Inc. (Cleveland, OH 44122), software was used. For each organ the values were calculated and recorded with standard error of mean (SEM). In the bladder, the areas with tumor were excluded. Radioactivity in one mL blood samples was counted together with ^{64}Cu -chloride standard prepared at the time of injection.

Radical cystoprostatectomy

Within four to six weeks of PET imaging, patients had radical cystoprostatectomy, lymphadenectomy and urinary diversion as a standard of care. In one case, where VPAC PET scan was positive with bone lesions, bone biopsy was performed, as part standard of care. Final surgical pathology results were considered a gold standard and were correlated retrospectively, with PET imaging results.

Statistical analysis

Means and standard deviations were calculated to summarize continuous study variables and frequency counts with percentages shown to summarize categorical variables. Diagnostic accuracy of the PET image UBC test was summarized by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) calculated for the test against the gold standard histology UBC diagnosis, in each of the three sites – bladder, prostate, and lymph nodes. Each of these diagnostic statistics were accompanied by an exact Clopper-Pearson 95% compatibility interval (CI).[20]

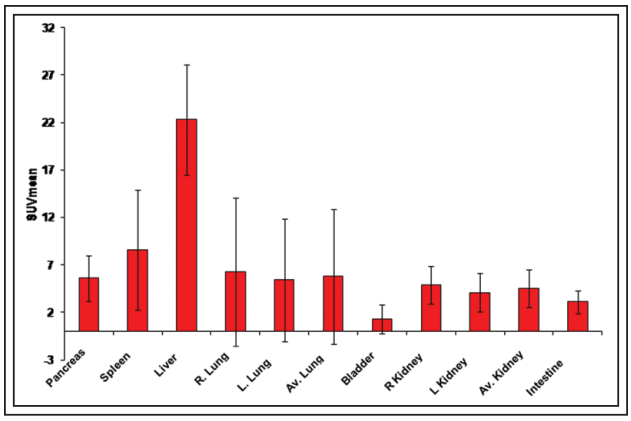

Figure 1. Tissue distribution of ^{64}Cu-TP3805 as determined by SUVmean on 19 patients (mean ± SEM).

Results

All patients completed PET imaging, and there were no adverse events. All patients also underwent successful surgery, with final surgical pathology available for comparison as the gold standard. Urinary excretion of radioactivity was negligible. The radioactivity in the liver, pancreas, renal cortex (but not in the medulla) and GI was apparent in all PET images. The tissue distribution of ^{64}Cu-TP3805 with SUVmean values is plotted in Figure 1. The liver was the target organ with SUVmean 22.3 ± 5.8 and that the normal bladder had the least SUVmean of 1.3 ± 1.1.

Patient demographic data together with the PET imaging results (SUVmax ±SEM) and the corresponding histological findings as reported independently by the pathologists, unaware of the PET imaging results, are presented in Table 1.

Sixteen of 19 patients (84%), received a full course of standard of care neoadjuvant chemotherapy prior to ^{64}Cu-TP3805 PET imaging and underwent radical cystoprostatectomy, pelvic lymphadenectomy and urinary diversion, Table 1. As confirmed by post-surgical tissue histology, VPAC PET images were true positive (TP) for UBC in 11 patients, true negative (TN) in 4, false positive (FP) in one and false negative (FN) in 3. The estimated diameter of lesions in the 3 FN patients was < 1.0 mm, which is less than the intrinsic resolution of PET scanner. Example of TP and TN, UBC images are given in Figure 2. Of the 11 patients with TP for UBC, nine received standard of care neoadjuvant chemotherapy regimen prior to PET imaging and two did not. The SUVmax for these lesions ranged from 1.8 ± 0.2 to .8.9 ± 3.1. In this pilot investigation, with a small number of study population, sensitivity for imaging UBC with ^{64}Cu-TP3805 VPAC was 79%, (95%, CI 49%-

TABLE 1. **Patient demographics, imaging results/histology**

Patient #	Age	Received	Bladder			Prostate			Lymph nodes		
/*Smoker	Yes	Treatment	PET	SUVmax	Histology	PET	SUVmax	Histology	PET	SUVmax	Histology
1*	57	Yes	-	-	+	-	-	-	-	-	-
2	58	Yes	+	8.9 ± 3.1	+	+	R 3.9 ± 0.5 L 4.1 ± 0.6	+ +	-	-	+
3	71	Yes	+	5.1 ± 2.2	+	+	2.4 ± 0.3	+	+	3.9 ± 0.2	+
4*	56	Yes	-	-	-	+	-	-	-	-	-
5*	59	Yes	-	-	-	+	R 1.9 ± 0.02 AP 2.7 ± 0.4	-	-	-	
6*	78	Yes	+	2.9 ± 0.5	+	+	R 4.1 ± 0.4 L 3.9 ± 0.3	-	-	-	+
7	65	Yes	+	2 ± 0.4	+	+	3.6 ± 0.4	-	-	-	-
8	44	Yes	+	3. 3± 0.5	+	+	2.9 ± 0.2	+	-	-	+
9*	65	Yes	+	3 ± 0.5	-	+	R 3.4 ± 0.3 L 2.05 ± 0.2	-	-	-	-
10*	55	Yes	+	3.95 ± 0.4 +	+	+	R 4.3 ± 0.3 L 3.7 ± 0.4	-	-	-	-
11*	50	Yes	+	2.1 ± 0.2	+	-	-	-	-	-	-
12*	80	Yes	-	-	+	+	2.3 ± 0.2	+	-	-	-
13	75	No	-	-	+	+	1.5 ± 0.1	+	-	-	-
14	84	Yes	+	1. 8± 0.2	+	+	1.6 ± 0.1	+	-	-	-
15	71	Yes	-	-	-	-	-	-	-	-	+
16*	72	No	+	3.14 ± 0.3	+	+	3.6 ± 0.2	+	-	-	-
17*	68	Yes	+	1.9 ± 0.2	+	+	5.4 ±	+	-	-	-
18*	66	No	+	3.1 ± 0.3	+	-	-	-	-	-	-
19*	75	Yes	-	-	-	-	-	-	-	-	-
				TP 11 TN 4 FP 1 FN 3			TP 8 TN 6 FP 5 FN 0			TP 1 TN 14 FP 0 FN 4	

* = smoker; + = positive; - = negative; R= right; L = left, Ap = apex; TP = true positive; TN = true negative; FP = false positive; FN = false negative

95%), specificity 80% (95%, CI 28%-100%), and has PPV and NPV were 92%, (95% CI 62%-100%) and 57% (95% CI 18%-90%), respectively. These values were greater than those expected for MRI or ^{18}F- FDG PET imaging.

When examining the prostate involvement from UBC, the PET uptake was TP in 8, TN in 6 patients and FP in 5. Out of 8 TP, 6 had neoadjuvant chemotherapy, and two did not. Out of the FP images in 5 patients, reexamination of histology results revealed incidental prostate cancer in 3, high grade prostatic intraepithelial neoplasia (HGPIN) in one and a prominent verumontanum in another. Results of these retrospective analyses are neither included in Table 1, nor are they considered in statistical analysis. PET images of three patients with incidental prostate adenocarcinoma are shown in Figure 3. For the entire cohort, the accuracy of detecting prostate adenocarcinoma revealed 100% sensitivity (95% CI 63%-100%), 55% specificity (95% CI 23%-83%), 62% PPV (95% CI 32%-86%), and 100% NPV (95% CI 54%-100%).

For lymph nodes (LN), images were TP for 1 LN, 6mm in size and with SUVmax 3.9 ± 0.2. LN images were TN in 14 and FN in 4 patients. For the 4 patients with FN LN images, the LN measured 2.9 mm, 3.5

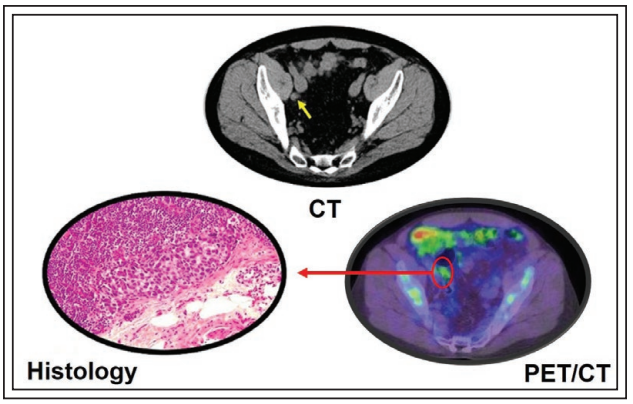

Figure 2. Figure depicts the only positive LN (SUVmax 3.9 ± 0.2, confirmed by histology) in a 44-year-old UBC patient treated with Methotrexate, Vinblastine, Doxorubicin, Cisplatin (MVAC) and Neulasta. The greater uptake of radioactivity seen above the LN may be the cecum.

mm, 1.5 mm and 2.5 mm well below the intrinsic spatial resolution of PET. The apparent true LN imaging, in this small patient population, was 79%. The LN imaging sensitivity was 20% (95% CI 1%-72%), specificity 100% (95% CI 77%-100%), PPV 100% (95% CI 3%-100%), and NPV 78% (95% CI 52%-94%), Figure 4.

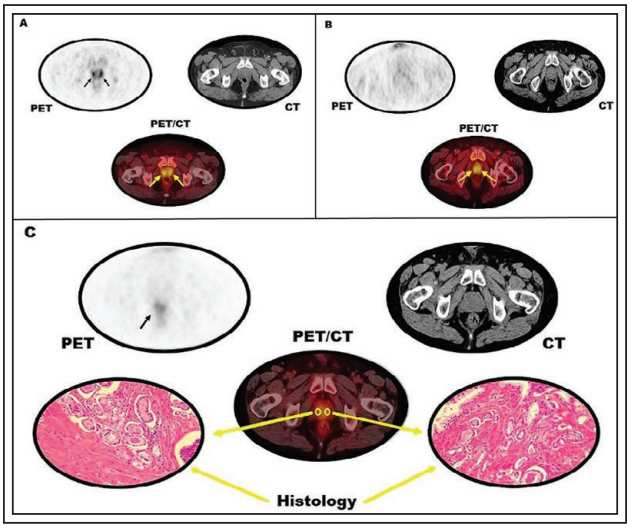

Figure 3. A, B and C are PET, CT and PET/CT images of 3 separate patients (44 yrs to 78 yrs). Patients A and B were treated with 4 and 3 cycles respectively of Cisplatin, Gemcitabine and Pembrolizumab and patient C with one cycle of Methotrexate, Vinblastine, Doxorubicin and Cisplatin (MVAC). All three had bilateral prostate adenocarcinoma (SUVmax 2.5 to 4.1). Post-surgical histology confirmed the disease (Gleason 3+3 each.)

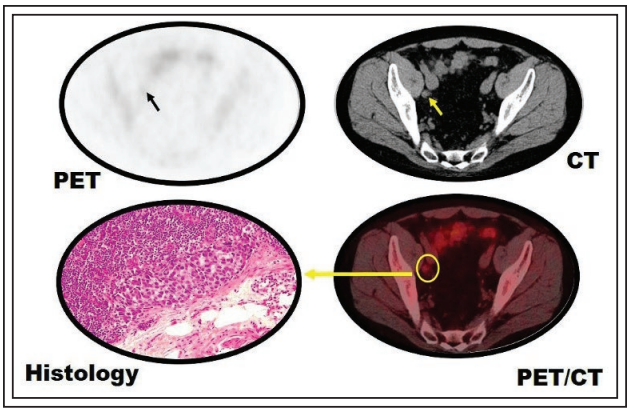

Figure 4. Depicts the only true positive (as confirmed by histology) lymph node in a 44-year-old UBC patient treated with Methotrexate, Vinblastine, Doxorubicin, Cisplatin (MVAC) and Neulasta. SUVmax was 2.1.

Consistent with the ^{99m}Tc-MDP (Methylene Di Phosphonate) bone scan obtained 5 weeks previously, PET image was TP in a patient with several bone lesions in iliac crest and spine. Biopsy of iliac crest confirmed metastasis for prostate adenocarcinoma, Figure 5. The patient was treated for prostate cancer previously, with intensity modulated radiation therapy (IMRT).

Among the patients, there were 12 smokers (0.5 to 2 packs/day for 30 to 40 yrs.) and 7 non-smokers. In all smokers there was either diffuse or focal pulmonary uptake of radioactivity, with no matching lesions seen on corresponding CT images. The probable reasons for this are discussed later. In 7 non-smokers,

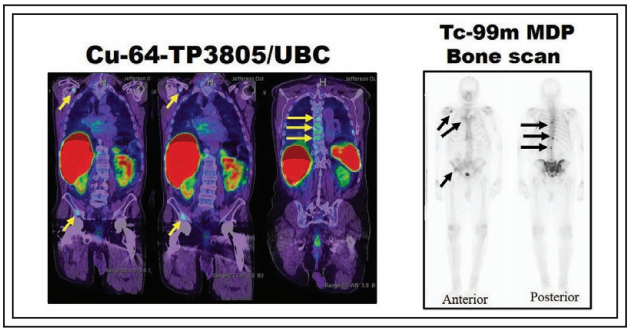

Figure 5. A 84 yr.prostate cancer male, treated with IMRT and Lupron with casodex, (PSA, 2.7 ng/mL), was diagnosed with malignant neoplasm of urinary bladder. ^{64}Cu-TP3805 image was positive for prostate and invasive bladder cancer. ^{99m}Tc-MDP (ant./ post.) bone scans performed 5 weeks previously are shown in the right and three coronal slices of PET/CT on the left. Post treatment bone lesion SUVmax were only ~ 2,but correctly correspond to the bone scan lesions.

3 patients (43%) had lung uptake. Out of these 3, one had 4 mm granuloma (as seen by non-contrast chest CT, R-SUVmean 14.4 ± 2.9, L-SUVmean 15.0 ± 3.1), one had 6 mm nodules in both lungs (R-SUVmean 24.4 ± 4.6 and L-SUVmean 28.3 ± 4.8) and one had clear lungs (R-SUVmean 7.1 ± 1.9, L-SUVmean 7.1 ± 1.8). The remaining 4 non-smokers had no reportable lung uptake.

Discussion

There is a paucity of novel imaging for UBC, and poor and inconsistent performance of conventional CT, MRI and FGD PET for the accurate evaluation of locally advanced and metastatic UBC.

In an attempt to address the unmet need in UBC, we investigated a first in human, PET imaging approach in which we targeted VPAC receptors expressed abundantly on all UBC types, irrespective of their heterogeneity.[11,12,19] VPAC receptors belong to the super-family of G protein and are expressed in high density on many types of cancer cells at the onset of oncogenesis.[12,21,22] On stroma, normal cells and benign masses, VPAC is minimally present. The high density of VPAC on malignant cells, and the low density on normal cells make VPAC a target to image UBC and therefore, worthy of a clinical investigation. The innovation of this approach lies in the fact that never before have the VPAC receptors, been targeted for imaging UBC, either in experimental animals or in humans, although they have been known to be upregulated in UBC for decades.

The imaging agent, ^{64}Cu-TP3805, designed, characterized and validated in our laboratory has several distinct virtues that are seemingly favorable for this application. In particular, ^{64}Cu- TP3805 has high VPAC receptor specificity (3.1×10^{-9}M), in vivo stability, negligible urinary excretion, and rapid blood clearance that permit imaging malignant lesions rapidly and accurately.[17] The half- life of ^{64}Cu (12.7 hrs.) is long enough to be able to dispatch it in any part of the country and to image patients without excessive radioactivity decay.

All experimental VPAC PET images were correlated with a gold standard histology performed independently by pathologists on tissues, extirpated at radical cystoprostatectomy, pelvic lymphadenectomy and urinary diversion. Out of 19 patients, UBC was PET positive in eleven and PET negative in 4 patients as confirmed by post-surgical histology (79%). These results although preliminary, are encouraging as they demonstrate better UBC imaging sensitivity, than either with MRI or ^{18}F- FDG PET/CT.[7,23,24] Generally SUV was higher for aggressive muscle invasive UBC. In one of the remaining four patients, SUVmax was 3 ± 0.5, yet histology was negative and PET image was considered false positive. Reasons for this FP are not clear. There were 3 FN patients with estimated UBC lesion size of < 1.0 mm, well below the intrinsic resolution of PET scanners.

In this group of a small number of patients, only one positive lymph node (~ 6 mm), in one patient, was PET imaged and was confirmed to be malignant by histology. In 14 patients, lymph nodes were TN, both by imaging and by histology. In remaining four patients, lymph nodes, < 3.5 mm in size, were malignant by histology but were not detectable, may again, be due to the limited spatial resolution of PET.

Approximately one third of patients who undergo radical cystectomy harbor prostatic involvement of urothelial carcinoma.[4] In this group of patients, prostate tracer uptake was TP in 8 (42%), TN in 6 (31%) and FP in 5 (26%). Reexamination of histological slides combined with digital autoradiography of the FP 5 patients revealed three incidental prostate adenocarcinoma, one HGPIN, and one prominent verumontanum. These results are the testimony of the sensitivity of the VPAC targeting approach and are consistent with our previous observations in imaging prostate cancer in human subjects.[18]

For quantification of tissue distribution in normal organs SUVmean values were calculated as this mode of quantification allows one to calculate the values from multiple voxels and help reduce the effect of image noise.[22,25] Generally the liver uptake in all patients was high.[15] There was also focal or diffuse lung uptake of the tracer in 12 patients, all of whom were smokers. Remaining seven patients had never smoked. Four of the seven non-smokers had no lung uptake but three did. Of these three, non-contrast CT scan revealed that one patient had granuloma (R-SUVmean 14.4 ± 2.9, L-SUVmean 15.0 ± 3.10) and the other two had emphysema (R-SUVmean 24.4 ± 4.6, L-SUVmean 28.3 ± 4.8 and R-SUVmean 7.1 ± 1.9, L- SUVmean 7.1 ± 1.8, respectively). Literature suggests that in smokers with or without bronchitis, emphysema or chronic obstructive pulmonary disease (COPD), alveolar cells express VPAC receptors which may have led to the uptake of ^{64}Cu-TP3805.[26,27] The increased lung uptake in the 12 patients who smoked may also be consistent with the expression of VPAC receptors. Importantly, however, none of these patients experienced any adverse events or pulmonary symptoms despite the lung parenchymal uptake of the VPAC tracer.

Never before have been the VPAC receptors targeted for PET imaging of UBC. The high density VPAC expression on UBC cells, combined with the lack of urinary excretion of ^{64}Cu-TP3805 make the VPAC

targeting approach especially appealing to image UBC. While sample size is small, the data suggest that targeting VPAC receptors enables to image UBC, as well as the bony metastases, prostate involvement and the malignant lymph nodes, all in one PET/CT scan. No other single modality, radiologic, scintigraphic or optical, associated with the management of UBC patients has this capacity. The approach to target VPAC using of ^{64}Cu-TP3805 to image UBC is therefore promising and should be evaluated in in other patient cohorts. There are several other areas of unmet need for accurate molecular imaging of UBC, which could evaluated in additional studies of VPAC PET imaging. This includes imaging before and after systemic treatment to determine response to therapy; at initial diagnosis in a treatment naïve cohort for accurate staging; and in the setting of suspected metastasis to guide biopsy and treatment. VPAC PET imaging may contribute to the management of patients with UBC.

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Conflict of interest: Mathew Thakur, PhD, holds a US patent on the Cu-64-TP3805 PET imaging technology, licensed to NuviewDx. He is consultant to NuviewDX and KOP therapeutics Inc. Leonard Gomella, MD, reports conflicts of interest, the entities are Astellas, Abbvie, Astra Zeneca, Bayer, Ferring, Merck, Clovis and MDx Health: advisory board. Jean Hoffman-Censits, MD reports conflicts of interest, the entities are Genetech, Foundation Medicine: research support, Seattle Genetics: paid advisory board, and Astra Zeneca: paid consultant. Edouard Trabulsi, MD, reports conflicts of interest, the entities are Pfizer, Astellas, Janssen, and GenomeDx: personal fees. All other authors have no conflict of interest to report. □

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