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VAPC-1 Targeted ^{64}Cu -TP3805 kit preparation and its evaluation

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Abstract

Introduction—Previously, our laboratory has shown that ^{64}Cu -TP3805 can specifically target VPAC-1 receptors and be used for Positron Emission Tomography (PET) imaging of breast (BC) and prostate cancer (PC) in humans. Present work is aimed at the formulation of a freeze-dried diaminedithiol-peptide (N_2S_2 -TP3805) kit and its evaluation for the preparation of ^{64}Cu labeled TP3805. Parameters such as pH, temperature and incubation time were examined that influenced the radiolabeling efficiency and stability of the product.

Methods—Kits were prepared under different conditions and radiolabeling efficiency of TP3805 kit was evaluated for a range of pH 3.5–8.5, after addition of ^{64}Cu in 30 μl , 0.1M HCl. Incubation temperature (37–90°C) and time (30–120min.) were also investigated. Kits were stored at –10°C and their long term stability was determined as a function of their radiolabeling efficiency. Further, stability of ^{64}Cu -TP3805 complex was evaluated in presence of fetal bovine serum and bovine serum albumin by using SDS polyacrylamide gel electrophoresis. Kits were then used for PET imaging of BC and PC following eIND (101550) and institutional approvals. Specificity of ^{64}Cu -TP3805 for VPAC-1 was examined with digital auto radiography (DAR) of prostate tissues obtained after prostatectomy, benign prostatic hyperplasia (BPH) tissue, and benign and malignant lymph nodes. Results were compared with corresponding tissue histology.

Results—Radiolabeling efficiency was 95% at final pH ~7.2 when incubated at 50°C for 90 min. Kits were stable up to 18 months when stored at –10°C, and ^{64}Cu -TP3805 complex exhibited excellent stability for up to 4h at room temperature. ^{64}Cu -TP3805 complex did not show any

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transchelation even after 2h incubation at 37°C in 10% FBS as well as in BSA as determined by SDS PAGE analysis. DAR identified 95% of malignant lesions 11 new PC lesions, 20 high grade prostatic intraepithelial neoplasia, 2/2 ejaculatory ducts and 5/5 urethra verumontanum not previously identified. The malignant lymph nodes were correctly identified by DAR and for 3/3 BPH patients, and 5/5 cysts, DAR was negative. In human BC (n=19) and PC (n=26) were imaged with 100% sensitivity.

Conclusion—Availability of ready to use N₂S₂-peptide kits for ⁶⁴Cu labeling is convenient and eliminates possible day to day variation during its routine preparation for clinical use.

Keywords

Kit formulation; Positron emission tomography; ⁶⁴Cu; kit; TP3805; prostate cancer; breast cancer

1. Introduction

There has been an increasing interest in receptor mediated radiolabeled peptides for positron emission tomography (PET) or single photon emission computerized tomography (SPECT) imaging in basic, translational and clinical research [1–3]. For PET imaging, recent literature advocates the use of ⁶⁴Cu [*t*_{1/2} 12.7h, β[−] 37.1%, β⁺ 17.9%]) because of its well-established chemistry [4] and characteristics that allow for both imaging and potentially, in large quantity, for targeted radiotherapy [5]. Furthermore due to the presence of its crystal-field stabilization energy, ⁶⁴Cu possesses well-characterized chelation methods [6–8] and is generally less prone to ligand exchange.

However the selection of a bifunctional chelating agents (BFCA) one of the most important criteria for its *in vivo* stability [9–11]. The majority of BFCA's used for the chelation of "⁶⁴Cu" have centered on tetraaza-based macrocycles (i.e. DOTA, TETA) [8, 12]. However, their resultant image quality is often less than optimal either due to slow release of ⁶⁴Cu by decomposition of the complex in blood, transchelation in the liver or to excessive bladder uptake resulting from urinary excretion and resulting into elevated uptake in non-targeted tissues such as the liver, kidneys and bladder [13–18].

N₂S₂ type diaminedithiol ligands form a stable, neutral and planar complex with Cu²⁺ ions and permits radiolabeling at physiological pH [19]. Previously we accomplished ⁶⁴Cu chelation was accomplished by synthesizing protected diaminedithiol (N₂S₂)-VPAC1 analog (TP3805) [20] and successfully evaluated VPAC-1 receptors specific imaging in xenograft animal tumor model and transgenic mice for breast and prostate cancer [21–24]. Blood clearance and *in vivo* stability ⁶⁴Cu-TP3805 complex through urine were also investigated. Following FDA and IRB approvals the use of ⁶⁴Cu-TP3805 was translated into PET imaging of breast [20] and prostate cancer [25] imaging in human.

To make it convenient for reliable and routine preparations, we developed a kit for ⁶⁴Cu labeling of TP3805. In the present work, detailed studies have been performed on the effect of various parameters such as pH, temperature and incubation time on the labeling efficiency, interaction with bovine serum albumin (BSA) as well as stability of kit at −10°C. A procedure for the conjugation of ⁶⁴Cu-TP805 with 95% radiochemical purity has been

achieved by using the instant freeze-dried kit. The kit formulation is evaluated in breast cancer [20], as well as prostate cancer PET imaging [25] and prostate cancer histological tissue slices obtained using excised whole mount human prostate tissue. Correlation between the experimental findings and histological results has been excellent.

2. Material and methods

2.1 Reagents

TP3805, a 28-amino acid peptide, was custom synthesized by American Peptide Co. (Sunnyvale, CA) on a Wang resin, purified and characterized by electrospray mass spectroscopy [21]. The peptide stock (Molecular weight = 3805D) was stored at -80°C until use. Stannous chloride and sodium hydroxide were purchased from Thermo Fisher Scientific (Pittsburgh, PA), Hydrochloric acid (HCl), glucoheptonate and glycine was purchased from Sigma-Aldrich (St. Louis, MO). ^{64}Cu , produced by $^{63}\text{Ni}-(p, n)$ reaction using a cyclotron, was obtained from Washington University (St. Louis, MO). Fetal bovine serum (FBS), bis-tris protein gel, coomassie blue, protein standard, LDS sample buffer and bovine serum albumin (BSA) were procured from Life-Technologies (Pittsburgh, PA) and soy broth was purchased from (Becton Dickinson, Franklin lake, NJ).

2.2 Effect of pH and peptide quantity on the radiochemical quality of ^{64}Cu -TP3805

In order to understand the impact of pH on radiolabeling yields, kits were formulated with 0.2M glycine buffer at various pH 6.5, 7, 8, 9.27 and 10 while the ratio of other ingredients were kept the same as in kit preparation section, including the addition of total volume of ^{64}Cu (30 μl , 0.1M HCl).

Kits were also made with varying amounts of peptide Fig.1 (5, 10, 15, 20 and 25 μg), using increasing amounts of the stock solution 4 $\mu\text{g}/\mu\text{l}$ in 0.1 M Sodium acetate at pH 4.5. To these kits, the amount of ^{64}Cu was added in 30 μl , 0.1M HCl and all other kit ingredients were constant as described in kit preparation section, regardless of the amount of peptide used.

2.3 Effect of temperature and incubation time on radiolabeling efficiency of kit

In order to, study the effects of temperature and incubation time on ^{64}Cu radiolabeling efficiency, TP3805 kits were incubated at various temperatures (37, 50, 70 and 90°C) and time (30, 45, 60, 90 and 120 min.). After radiolabeling, the radiochemical purity of the complexes was determined with radio-HPLC.

2.4 Reducing agent

To achieve the maximum radiolabeling efficiency and stable complex, it is important to reduce the 2 benzyl groups from the chelating agent. For this purpose we evaluated multiple mild reducing agents (ascorbic acid, sodium cynoborohydride and stannous chloride). In all of them 200 μg stannous chloride in 1mg Glucoheptonic acid worked best for 20 μg of TP3805. Glucoheptonic acid worked as a solvent for stannous chloride as well as it protect the cells against genotoxic action of tin(II) [26].

2.5 Kit preparation

Using the optimal conditions determined from the above parameters a single step freeze dried kit was formulated using 0.2M glycine buffer (pH 9.27). Before kit preparation, all glassware, such as 10mL vials and test tubes, were washed with 6M HCl, rinsed thoroughly with deionized water and oven dried. Pipette tips, rubber caps and aluminum sealing caps were also sterilized. All solutions were made in deionized water. Ten mg of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 1mL glucoheptonate (50 mg/mL) solution and filtered through 0.22 μm cellulose membrane syringe filter (Millipore syringe filter, USA). In a 10 mL sterile glass vial, 20 μg of TP3805 was added (4 $\mu\text{g}/\mu\text{L}$ 0.1M Sodium acetate buffer, pH 4.6), followed by addition of 20 μL of SnCl_2 /glucoheptonate solution as prepared above and 200 μL of 0.2M glycine buffer (pH 9.27) were added, vortexed for 10 sec. to this mixture, and freeze dried by placing the vials in a dry ice-acetone bath. The vials were then lyophilized for 12–14h at -10°C and 300mT (Bench top pro, SP scientific, Warminster, PA) and sterile nitrogen gas was introduced in the chamber. The vials were then sealed, documented appropriately, dated and stored at -10°C until use. Radiolabeling efficiency of kit for ^{64}Cu , and its stability were analyzed by using radio-high-performance liquid chromatography (radio-HPLC, Shimadzu Corp. Tokyo, Japan) and one or more of them were tested for sterility by using soy broth medium at 37°C before using in patients.

Long-term stability of kits stored at -10°C , was also checked by radiolabeling yield with ^{64}Cu for up to 18 months. Whenever needed, kit content was labeled with ^{64}Cu by adding 30 μL 0.1M HCl containing the required quantity of ^{64}Cu and heated at 50°C for 90 min. The radiolabeling efficiency was then determined by radio HPLC analysis. The integrity of peptide was also confirmed after heating by using MALDI/TOF.

2.6 ^{64}Cu -TP3805 conjugation

On the day of preparation, the kit vial was kept at room temperature (RT) for 30–45 min before use. Labeling was performed with the addition of a required quantity of ^{64}Cu solution in 30 μL of 0.1M HCl, followed by 220 μL of sterile deionized water. The reaction mixture was vortexed and incubated at 50°C for 90 min. After incubation, the radiochemical purity was determined by radio-HPLC, using a reverse phase Zorbax 300SB.C18, 4.6mm $\times$ 250mm column (Schimadzu, Columbia, MD), and a gradient solvent (from 0% to 100% acetonitrile in 0.1% aqueous trifluoroacetic acid (TFA) in 7 min and then to 90% in 15 min) ran for 23 min. Labeling efficiency of 95% was considered the passing criterion for the kit.

2.7 ^{64}Cu -TP3805 complex stability

Ex vivo stability of ^{64}Cu -TP3805 complex were performed in 3 different ways. To determine the stability of ^{64}Cu -TP3805 complex, the solution was stored at RT and followed by radio-HPLC analysis at each one-hour interval for up to 4h.

The result of interaction of radiolabeled ^{64}Cu -TP3805 with bovine serum albumin (BSA) was also examined as reported in our previous study [21]. Briefly, ^{64}Cu -TP3805 complex (10 μL), was mixed with BSA (2 μL , 50mg/mL) followed by incubation at 37°C for 2h. After completion of incubation, 4 μL loading buffer was added [NUPAGE, LDS sample buffer] followed by incubation at 100°C for 5 min. Final volume for all the samples was 20 μL , which

was then analyzed on a 12% denaturing SDS-polyacrylamide gel. The gel was run for 60 min at 160V and stained with coomassie blue (Fermentas Waltham, MA) to visualize BSA. The gel was also processed with Kodak image station 4000 R to analyze the % ^{64}Cu associated with BSA.

Stability of ^{64}Cu -TP3805 was also checked with FBS. Briefly, 100 μl of ^{64}Cu -TP3805 was incubated with 10% FBS for 30, 60 and 120 min at RT. 5 μCi of ^{64}Cu -TP3805 was loaded on thin-layer chromatography (ITLC-SG; Gelman Sciences, Ann arbor, MI) and aliquots were analyzed by TLC with a mixture of 10% $\text{NH}_4\text{OAc}:\text{MeOH}$ (3:7) as a mobile phase for labeling percentage. The strips were cut and activity was counted by gamma counter (PerkinElmer, Waltham, MA).

2.8 Sterility test

Approximately 100 μl of the ^{64}Cu -TP3805 solution ready for injection, were added aseptically to 10mL tryptic soy broth and stored in a humidified, 5% CO_2 incubator at 37°C. The test tube was observed daily for 7 days to detect any turbidity or microbial growth.

2.9 Digital autoradiography (DAR)

The purpose of this study was to determine the specificity of ^{64}Cu -TP3805 for VPAC-1 receptors. We performed the DAR on 132 de-paraffinized, 3 μm thick, histological sections prepared from whole mount prostate tissue excised from 11 PC patients. Histological sections were incubated at RT for 90 min with ^{64}Cu -TP3805 (10–15 μCi) solution, washed thoroughly with phosphate buffered saline (PBS), dried and subjected to DAR (Imaging 4000, Kodak/Bruker Inc. Rochester, NY) for 15 sec. These slides were then stained with hematoxyline and Eosin (H&E) and foci were marked by an attending pathologist unaware of the DAR results as PC, benign, cyst, or prostatic intraepithelial neoplasia (PIN). In addition, three histological slices, each from 3 patients, with benign prostatic hyperplasia (BPH) (n=9), from one malignant lymph node (n=3) and from one benign lymph node (n=3) were examined with DAR followed by H&E staining. The histological findings were then analyzed with the autoradiographic findings for match and mismatch.

3. Results

The kits were formulated in a verity of ways to examine how these changes might affect the radiolabeling. The optimum kit formulation for the conjugation of ^{64}Cu contains 1mg glucoheptonate and 200 μg of SnCl_2 , 200 μl of 0.2M glycine buffer pH 9.27 and lyophilized at low temperature (-10°C).

To determine the effect of pH on the radiolabeling yield of kit, we formulated the kits at various pH i.e. 6.5, 7.0, 8.0, 9.27 and 10, of 0.2M glycine buffer. Radiolabelling efficiency and the final pH of kit was analyzed after adding ^{64}Cu in 0.1M HCl 30 μl and 220 μl deionized water. Which rendered final pH was ~3.0, ~3.5, ~6.0 ~7.2 and ~8.7 respectively, with radiolabeling efficiency 8% $\pm$ 2%, 10% $\pm$ 2.7%, 45% $\pm$ 2.9%, 97% $\pm$ 2.5% and 90% $\pm$ 2.1% at respectively (Fig. 2A). Radiolabeling yields were analyzed by radio-HPLC and pH was analyzed by using calibrated pH paper.

We further observed that at above condition, the radiochemical yield 95% could be obtained using 15µg of TP3805 (Fig. 2B). However chose to use 20µg of the peptide in the final kit formulation to eliminate any variation and to obtain consistently high radiolabeling yield.

Use of reducing agent (SnCl_2) is one of the important step in kit preparation and we found that 200µg of stannous chloride in 1mg glucoheptonic acid (data not shown) was the best concentration to achieve the desired radiolabeling.

The temperature optimization studies showed that radiolabeling yield increase with elevated temperature and attained maxima at 50°C to yield 95% after 90 min. of incubation time. No significant change was observed in radiolabeling yield above 50°C (Fig. 3A).

Similarly the radiolabeling efficiency of ^{64}Cu increased with increasing incubation time at constant temperature (50°C) and attained its maximum at 90 min (~95%) (Fig. 3B). No significant increase in conjugation was observed after increasing the incubation time above 90 min.

^{64}Cu -TP3805 was then prepared by adding ^{64}Cu in 30µl of 0.1M HCl, 200µl deionized sterile water followed by incubation at 50°C for 90 min. Radiochemical Purity of ^{64}Cu -TP3805 preparation as determined by radio-HPLC was always 95%. The analytical data obtained using radio-HPLC for ^{64}Cu -TP3805 and free ^{64}Cu are presented in Fig. 4A.

The Specific activity of the ^{64}Cu -TP3805 kit preparation we have used is 2.85Ci/micromole. However depending upon our need, upto 13.0Ci/micromole Specific activity can be achieved since up to 70mCi ^{64}Cu is available in 30 microliter 0.1M HCl from Washington University, St. Louis.

The kit remained stable for 18 months (stored at -10°C), the longest time studied. All the kits complied with self-determined criteria of radiochemical purity (95%). The MALDI/TOF of TP3805 after heating (Fig. 4B) shows that molecular weight (3805D) of the peptide did neither change during the kit formulation (3A), nor did it alter after incubation at elevated temperatures (50–90°C) (3B). This data are consistent with our previous reports [24].

Interactions of radiopharmaceuticals with serum proteins under *in vivo* conditions are known to reduce stability increase uptake in normal tissues and reduced uptake in the targeted tissue. Fig. 4A demonstrates protein bands and the corresponding autoradiograph of SDS-PAGE analysis in which ^{64}Cu -TP3805 was incubated at 37°C for 2h with BSA. The quantitative PAGE data showed that 98% of ^{64}Cu was associated with TP3805 and negligible amount was transchelated to BSA protein, even after 2h incubation Fig. 5A.

Consistent with these data Fig. 5B demonstrates that ^{64}Cu -TP3805 had no transchelation to 10% FBS even after 2h incubation. ^{64}Cu -TP3805 appeared at Rf 0.01 and free ^{64}Cu migrates at Rf 1.0.

DAR for histological tissue examination is a well-established technique [27]. DAR data from 11 patients using 132 histological slides, each of which were H&E stained post-DAR,

and read by attending pathologist, who marked the lesions with a color code, and compared visually, provided better insight into the greater number of malignant lesions seen on PET images by DAR with >1.0 SUV than determined by histology of core biopsy [25].

Results given in Table 1 show that, in 132 slices, there were a total of 180 PC foci, as determined by histology. Out of these, 171 (95%), were identified by DAR. DAR missed 9 (5%) PC lesions due to technical artifact (tissue folding on the slides). Additionally, 11 small PC lesions (6.1%), previously unmarked by histologist were identified by DAR. Furthermore, DAR identified 20 lesions corresponding to high grade prostatic intraepithelial neoplasia (HGPIN), 2/2 ejaculatory duct and 5/5 urethra verumontanum not previously noted by histology. For 3 BPH patients without PC and 5/5 cysts, DAR was negative. In this series of patients there were no known metastatic bony lesions and PET images did not delineate any such masses. DAR correctly identified the malignant lymph node and benign lymph node [25].

4. Discussion

A reproducible preparation, long shelf life with ready to use ability and in vivo stability are the characteristics of primary importance for any successful radiopharmaceutical for its routine clinical use. In the current study, we have established a simple and robust practical kit formulation for ^{64}Cu labeling and evaluated its characteristics for long term clinical applications. The benefits of this kit formulation include: i) single step preparation, ii) reproducible and high radiochemical purity and iii) long term shelf life allowing a widespread distribution. Our data demonstrate that the kits we prepared had a reproducible labeling efficiency of 95% upon incubation with a required quantity of ^{64}Cu in 30 μl of 0.1M HCl, at 50°C for 90 min. The MALD/TOF of TP3805 after heating showed that the peptide was intact. This together with its preparation at physiologic pH ~ 7.2 and isotonicity allowed us to administer the preparation (^{64}Cu -TP3805) without further purification. All preparations ($n=50$) were sterile. The kits stored at -10°C were stable with a shelf life of 18 months and preparations were stable at room temperature for 4h and more than 48hrs at -80°C . When challenged with endogenous proteins (BSA and FBS) we observed that there was no transchelation of ^{64}Cu onto the protein. These data are consistent with those obtained with our previous reports [21]

Following the US FDA (eIND) and institutional approvals, the kit preparations evaluated as above have been successfully used for translational research in PET imaging of breast cancer ($n=19$) [20] and prostate cancer ($n=25$) in humans [25]. There were no adverse events and all malignant lesions were imaged with 100% sensitivity. Examples of images obtained are shown in (Fig. 6A and B).

Naturally, the concentration of the peptide also affects the radiolabeling yields of the kit. Receptor Specificity (K_d value) and IC_{50} of TP3805 for VPAC1 have been calculated in our previous study and compared with native VIP₂₈ control peptide [23]. These were $3.3 \times 10^{-9}\text{M}$ and $5.3 \times 10^{-8}\text{M}$ respectively for TP3805 as compared to $9 \times 10^{-8}\text{M}$ and $15 \times 10^{-9}\text{M}$ for VIP₂₈. [23]. Since the number of receptor sites is usually limited and the unlabeled peptide molecules are inversely proportional to the specific activity of ^{64}Cu -

TP3805 preparation, it is desirable to have a high specific activity of peptide for efficient delineation of the targeted lesions. Low quantity and high radiolabeling yields of ^{64}Cu -TP3805 made it a suitable biomolecule for our targeted imaging.

The correlation of digital autoradiography (DAR) results with whole mount histologic examinations of the same slides (Fig. 7), showed that, ^{64}Cu -TP3805 imaging is able to identify all malignant lesions only, but also identified HGPINs, the ejaculatory ducts, and urethra verumontanum, but not uptake on BHP or benign cysts lesions. Detection of HGPIN is consistent with early expression of VPAC1 prior to the modulation of cell morphology. PIN is the precursor of PC [28]. There are approximately 115,500 new cases of PIN diagnosed each year in the USA, which warrant biopsy, and most of which develop PC [28–30]. Similarly, ejaculatory ducts and urethra verumontanum, in which PC cells exist, were also seen by DAR. DAR was positive for malignant lymph node, but was negative for normal lymph nodes. These data consistently demonstrated positive results for malignant lesions and negative with benign tissues. The results also indicated the high specificity of ^{64}Cu -TP3805 to image VPAC1 positive malignant lesions. The data are also consistent with our previous observations in which we have targeted VPAC1 biomarker to image breast cancer in humans (n=19) [20] and prostate cancer (n=26) using ^{64}Cu -TP3805 [25].

5. Conclusions

The objective of formulating single vial kit for ^{64}Cu labeling has been achieved. The single vial freeze dried kits, which can be radiolabeled with ^{64}Cu with a high degree of reproducibility, provide a convenient and reliable method for facile preparation of ^{64}Cu -TP3805 BPH DAR Histology Benign Prostate Tissue, “101-4P” for clinical use. The efficiency of labeling of TP3805 is governed by temperature, pH, stannous ion and time of incubation. Awareness of these factors and controlling them, when possible, has resulted in a highly promising radiopharmaceutical for PEM and PET imaging of BC and PC. We anticipated that the availability of freeze-dried kit formulation will expand the scope of VPAC-1 targeted ^{64}Cu -TP3805 for PET imaging of other malignant lesions such as those of lungs and bladder.

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Fig 1. Schematic presentation of TP3805. In the inset is provided a theoretical structure of ⁶⁴Cu-TP3805 after reduction of the two benzyl groups.

A. **B.****Fig. 2.**

A. ^{64}Cu -TP3805 radiolabeling yield at final pH 3–8.7 after addition of ^{64}Cu in 30 μl , 0.1M HCl.

B. Radio labeling efficiency with variable concentration of TP3805 after addition of ^{64}Cu in 30 μl , 0.1M HCl.

A. **B.****Fig. 3.**

A. ^{64}Cu -TP3805 radiolabeling yield at different temperature (37–90°C) incubated for 90 min. at final pH ~7.2 after addition of ^{64}Cu in 30 μl , 0.1M HCl.

B. ^{64}Cu -TP3805 radiolabeling yield with different incubation time (0–120 min.) at final pH ~7.2 after addition of ^{64}Cu in 30 μl , 0.1M HCl at 50°C.

Fig. 4.

A. Radio HPLC of $^{64}\text{CuCl}_2$ (elution time 3.6 min.) (A1) and that of ^{64}Cu -TP3805 5.5 min (A2).

B. MALDI/TOF- mass spectrum of TP3805 prior to any chemical modification (B1 and (B2) that of TP3805 after heating at 90°C. Data demonstrated chemical and thermal stability of TP3805.

A.

B.

Fig. 5.

A. Coomassie blue stained PAGE (A1) and Autoradiography (A2) analysis of ^{64}Cu -TP3805 Incubated with BSA for 2h at 37°C. Lane 1= molecular weight marker; Lane 2= BSA only; Lane 3= ^{64}Cu -TP3805 incubate with BSA; Lane 4= ^{64}Cu -TP3805. The radioactive bands have approximate MW of 3800D.

B. Stability of ^{64}Cu -TP3805 incubated for 120 min. at 37°C in 10% FBS

Fig. 6.

(A) 72 yr. old male with PC (Gleason score 7) received 4mCi ^{64}Cu -TP3805, 60 min. prior to PET/CT imaging. Images in different colors delineated PC. (B) 40 yr. old female with histologically proven invasive ductal carcinoma (ER-positive, PR-positive, HER2-negative) had positive 18F-FDG (B-right). Following complete decay of F18-FDG, 3.8mCi of ^{64}Cu -TP3805 were administered and sequential PEM images were obtained at 17 min and 2hr 17 min post administration (B center and right).

Fig. 7.

Examples of digital autoradiography (DAR) and hematoxylin and eosin stained tissues on the same slide (histology as marked by the pathologist) are shown for 3 different patients: A and B: Histology and DAR of prostate tissues of two separate patients with BPH. Note the absence of ^{64}Cu radioactivity consistent with normal histology. C): A patient with PC (Gleason 3+4) received $^{64}\text{CuTP3805}$ prior to radical prostatectomy. The malignant lesions are marked on histology slides and corroborate well with DAR.

Table-1

Demography of Eleven H&E[†]/Histology Patients and DAR^{*} Results

Patient	Age (yrs)	Race	Biopsy GLSN [§]	Pathology GLSN	Pathology Stage	SUV Max Range	H&E-PC Lesions	DAR Lesions	Additional DAR Lesions
1	59	C [‡]	3+4	3+3	T2cNO	3.2–5	9	9	HGPIN [?] = 2, Cyst = 3, NPC [¶] = 2
2	67	C	3+4	3+4	T2bNO	3–5.3	9	9	UV [#] = 1
3	74	C	3+4	3+4	T2cNO	0.4–1.9	13	12	UV = 1, ED ^{**} = 1
4	54	C	3+4	3+4	T3aNO	0.3–2.3	9	9	NPC = 3, HGPIN = 3
5	59	C	3+4	3+4	T2cNO	0.6–1.6	24	23	NPC = 2, HGPIN = 2, ED = 1
6	67	C	3+4	3+4	T3aNO	2.5–6.3	43	43	NPC = 2, HGPIN = 12, Cyst = 2, UV = 3
7	54	C	3+3	3+3	T3aNO	1.0–3.5	6	4	Cyst = 2
8	59	C	3+4	3+4	T2cNO	0.6–1.9	10	7	PAH = 2
9	56	C	3+4	3+4	T2cNO	1.5–5.3	19	19	PAH = 4,
10	57	C	3+4	3+4	T3aNO	0.5–2.4	19	17	NPC = 1, HGPIN = 1, Cyst = 3
11	58	AA [∞]	3+4	3+4	T3aNO	2.3–8.8	19	19	NPC = 1, UV = 1

^{*} Digital Autoradiography,

[†] Hemotoxylin & Eosin,

[‡] Caucasian,

[∞] African American,

[§] Gleason Score,

[?] High Grade Prostatic Intraepithelial Neoplasia,

[¶] New Prostate Cancer Lesion (identified by DAR),

[#] Urethra Verumontanum,

^{**} Ejaculatory Duct, Post atrophic Hyperplasia (PAH)