Receptor-targeted peptide conjugates based on diphosphines enable preparation of $^{99m}$Tc and $^{188}$Re theranostic agents for prostate cancer

Truc T. Pham,†* Ingebjørg N. Hungnes, † Charlotte Rivas, † Julie Cleaver, † George Firth, † Philip J. Blower, † Jane Sosabowski, † Gary J. R. Cook, † Lefteris Livieratos, †,‡ Jennifer D. Young, †,‡ Paul G. Pringle, ‡ Michelle T. Ma†*

†School of Bioengineering and Imaging Sciences, King’s College London, 4th Floor Lambeth Wing, St Thomas’ Hospital, London, SE1 7EH, UK

‡Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London, EC1M 6BQ, UK

‡Department of Nuclear Medicine, Guy’s and St Thomas’ Hospitals NHS Foundation Trust, Guy’s Hospital, London, SE1 9RT, UK

‡School of Chemistry, University of Bristol, Cantock’s Close, Bristol, BS8 1TS, UK

†Authors contributed equally to this study

*Corresponding authors: truc.pham@kcl.ac.uk (ORCID: 0000-0001-5850-4592), michelle.ma@kcl.ac.uk (ORCID: 0000-0002-3349-7346)

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Abstract

Background: Benchtop $^{99m}$Tc and $^{188}$Re generators enable economical production of molecular theranostic $^{99m}$Tc and $^{188}$Re radiopharmaceuticals, provided that simple, kit-based chemistry exists to radiolabel targeting vectors with these radionuclides. We have previously described a diphosphine platform that efficiently incorporates $^{99m}$Tc into receptor-targeted peptides. Herein we report its application to label a prostate specific membrane antigen (PSMA) targeted peptide with $^{99m}$Tc and $^{188}$Re for diagnostic imaging and systemic radiotherapy of prostate cancer.

Methods: Two diphosphine-dipeptide bioconjugates, DP1-PSMA(t) and DP2-PSMA(t) were formulated into kits for radiolabeling with $^{99m}$Tc and $^{188}$Re. The resulting radiotracers were studied in vitro, in prostate cancer cells, and in vivo, in mouse xenograft models, to assess similarity of uptake and biodistribution of each $^{99m}$Tc/$^{188}$Re pair of agents.

Results: Both DP1-PSMA(t) and DP2-PSMA(t) could be efficiently radiolabeled with $^{99m}$Tc and $^{188}$Re using kit-based methods to furnish isostructural compounds, M-DP1-PSMA(t) and M-DP2-PSMA(t), (M = $^{[99m]$Tc}$Tc, $^{[188]$Re}$Re). All $^{99m}$Tc/$^{188}$Re radiotracers demonstrated specific uptake in PSMA-expressing prostate cancer cells, with negligible uptake in prostate cancer cells that did not express PSMA or in which PSMA uptake was blocked. M-DP1-PSMA(t) and M-DP2-PSMA(t) also exhibited high tumor uptake (18 – 30 %ID/g at 2 h post-injection), low retention in non-target organs, fast blood clearance and excretion predominantly via a renal pathway. Importantly, each pair of $^{99m}$Tc/$^{188}$Re radiotracers showed near-identical biological behavior in these experiments.

Conclusions: We have prepared and developed novel pairs of isostructural PSMA-targeting $^{99m}$Tc/$^{188}$Re theranostic agents. These generator-based theranostic agents have potential to provide access to the benefits of PSMA-targeted diagnostic imaging and systemic radiotherapy in healthcare settings that do not routinely have access to either reactor-produced $^{177}$Lu radiopharmaceuticals or PET/CT infrastructure.

Key words: technetium-99m, rhenium-188, SPECT, phosphine, PSMA
Introduction

The PSMAt peptide, which targets the prostate specific membrane antigen (PSMA), has had clinical impact as a vector for delivering radionuclides to prostate cancer for diagnostic imaging and systemic Peptide Receptor Radionuclide Therapy (PRRT). The radiopharmaceutical [\(^{177}\)Lu]Lu-PSMA-617 (1,2) has recently been approved by the FDA for PRRT of metastatic castration-resistant prostate cancer. Diagnostic PET imaging with \([^{68}\text{Ga}]\text{Ga-PSMA-11}\) can inform clinical decision-making for treatment of prostate cancer, and \([^{68}\text{Ga}]\text{Ga-PSMA-11}\) is widely used as a diagnostic companion to \([^{177}\text{Lu}]\text{Lu-PSMA-617}\) (3). PSMA-targeted radiopharmaceuticals for SPECT/\(\gamma\)-scintigraphy imaging, such as \([^{99m}\text{Tc}]\text{Tc-MIP-1404,}(4,5)\) \([^{99m}\text{Tc}]\text{Tc-PSMA-IIS}\) (6,7) and \([^{99m}\text{Tc}]\text{Tc-EDDA/HYNIC-PSMA}\) (8,9) have been developed as alternatives to \([^{68}\text{Ga}]\text{Ga and PET are less accessible, but generator-based}\) \([^{99m}\text{Tc}]\text{Tc and SPECT/\(\gamma\)-scintigraphy cameras are available. Although these}\) \([^{99m}\text{Tc}}(t_{1/2}=6 \text{ h}, 90\% \gamma, 140 \text{ keV})\) radiotracers exhibit lower sensitivity compared to PSMA-targeted PET radiotracer alternatives, particularly in the case of biochemical recurrence of prostate cancer at low PSA levels or low tumor volume, they have potential utility in providing useful diagnostic information at high tumor volume, assessing suitability for and response to PRRT, and radioguided surgery where detection of every site of small volumes of disease is less critical (4,7,8).

A range of radiometal ions with therapeutically efficacious emission profiles (e.g. \(^{225}\text{Ac, 227}\text{Th, 212}\text{Pb and 161}\text{Tb}\)) have been used as alternatives to \(^{177}\text{Lu}\). However, in lower- and middle-income countries (LMICs), the availability of PRRT is limited by cost. The batch-produced radiopharmaceuticals that prevail in high income countries are prohibitively expensive and, in LMICs, are available only to the wealthiest patients. Additionally, the availability of PET infrastructure, including cyclotrons and scanners, in LMICs is limited (10).

The chemistry of rhenium (Re) is closely similar to that of its lighter congeners Tc. Importantly, \(188\text{Re}\) \((t_{1/2}=17 \text{ h}, 100\% \beta^-, 2.12 \text{ MeV}, 15\% \gamma, 155 \text{ keV})\) is available from a benchtop \(188\text{W/}^{188}\text{Re}\) generator, offering hospitals an economical and routinely accessible source of a therapeutic radionuclide. Radiopharmaceuticals based on the theranostic \(99m\text{Tc}/^{188}\text{Re}\) pair offer a potentially viable solution to economical and geographical barriers posed by existing theranostic PRRT (12–13). “Traditional” \(99m\text{Tc-radiopharmaceuticals are used in 30 million scans worldwide per year, including in LMICs, in combination with \(\gamma\)-scintigraphy (10,14). Additionally, international consortia have identified \(188\text{Re}\) as a highly promising basis for systemic radiotherapy in LMICs: recognizing the affordability of \(188\text{Re}\), the International Atomic Energy Agency has sponsored multinational clinical trials of \(188\text{Re-labeled Lipiodol for treatment of inoperable liver cancer in LMICs}\) (11). \(188\text{Re-labeled Lipiodol proved effective and inexpensive. \(188\text{Re-labeled bisphosphonates have also been extremely beneficial in palliative treatment of bone metastases in LMICs}\) (15). There is high potential for the combination of \(99m\text{Tc}\) with \(188\text{Re}\) to provide economical, population-wide access to stratified molecular imaging and PRRT in LMICs, provided that suitable chemical platforms are available to enable radiochemical production of well-defined pairs of theranostic \(99m\text{Tc}/^{188}\text{Re}\) agents.

A new theranostic pair of \(99m\text{Tc}/^{188}\text{Re}\)-labeled peptide radiotracers has recently demonstrated preclinical and clinical PSMA-targeting efficacy in prostate cancer (16), highlighting the potential utility of this theranostic approach. We have contemporaneously developed two kit-based \(99m\text{Tc-radiolabeled agents, \([^{99m}\text{Tc}]\text{Tc-DP1-PSMA}\) and \([^{99m}\text{Tc}]\text{Tc-DP2-PSMA}\) (Figure 1), targeting PSMA, and reported their chemical properties (17). These compounds are based on a diphosphine chelator (18), which also coordinates Re\(^v\); we have used analogous non-radioactive Re\(^v\) derivatives to chemically characterize the radioactive Tc\(^v\) compounds (17–19). Herein, we report (i) the preparation of isostructural and isoelectronic \(188\text{Re-labeled derivatives, \([^{188}\text{Re}]\text{Re-DP1-PSMA}\) and \([^{188}\text{Re}]\text{Re-DP2-PSMA}\), (ii) the biological behavior of the novel \(99m\text{Tc}\) radiotracers in prostate cancer models, and (iii) the comparative biological behavior of \(188\text{Re}\) compounds.

Materials and Methods

\(188\text{Re radiolabeling:} \) \([^{188}\text{Re}]\text{Re}\(^v\)-citrate was prepared from a saline solution of “pre-concentrated” (13) \([^{188}\text{Re}]\text{ReO}_4\) in >95% yield (20,21). An aliquot of this solution (75 \(\mu\)L) was added to a DP1-PSMA kit or DP2-PSMA kit (Table S1), which was heated at 90 \(^\circ\)C for 30 min. The reaction solution was then analyzed by C18 radio-HPLC (high performance liquid chromatography). \([^{188}\text{Re}]\text{Re-DP1-PSMA}\) eluted at 12.7 min and \([^{188}\text{Re}]\text{Re-DP2-PSMA}\) eluted at 17.5 min (Figure S1). The reaction mixtures, containing either \([^{188}\text{Re}]\text{Re-DP1-}
PSMA or $^{188}\text{Re}$Re-DP2-PSMAt were purified and reformulated for biological experiments (Supplemental Data).

$^{99m}\text{Tc}$ and $^{188}\text{Re}$ radiotracer uptake in prostate cancer cells: Solutions containing radiotracer (100 kBq, in 8-12 μL of PBS, > 95% radiochemical purity) were added to DU145-PSMA+ or LNCaP prostate cancer cells (22), and the cells incubated at 37 °C for 1 h. Non-specific uptake was determined by using non-PSMA expressing cells (DU145, PC3) or by blocking PSMA expressing cells (DU145-PSMA+, LNCaP) with PMPA (2-phosphonomethyl pentanedioic acid). After incubation, cells were washed and samples collected for radioactivity counting. Uptake and localization of $^{99m}\text{Tc}$ radiotracers was measured over time (15, 30, 60 and 120 min) in DU145-PSMA+ or LNCaP cells (Supplemental Data).

SPECT/CT scanning and biodistribution studies in mice: All animal experiments were ethically reviewed by an Animal Welfare & Ethical Review Board at either King’s College London or Barts Cancer Institute and carried out in accordance with the Animals (Scientific Procedures) Act 1986 UK Home Office regulations governing animal experimentation. Subcutaneous prostate cancer DU145-PSMA+ or DU145 xenografts were produced in SCID/beige mice (male, 7-12 weeks old). Subcutaneous LNCaP prostate cancer xenografts were produced in athymic nude mice (Crl:NU(NCr)-Foxn1nu, male, 6-7 weeks old). SPECT/CT and biodistribution studies using $^{99m}\text{Tc}$ and $^{188}\text{Re}$ radiotracers were performed once a tumor had reached an appropriate size (Supplemental Data).

Results

Preparation of $[^{188}\text{Re}]\text{Re-DP1-PSMA}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMA}$

We have previously prepared $[^{99m}\text{Tc}]\text{Tc-DP1-PSMA}$ and $[^{99m}\text{Tc}]\text{Tc-DP2-PSMA}$ using a single-step radiolabeling kit (17). Here, the analogous $^{188}\text{Re}$ agents were prepared in two steps from a saline solution containing $[^{188}\text{Re}]\text{ReO}_4^-$, obtained from a $^{188}\text{W}/^{188}\text{Re}$ Oncobeta generator. In the first step, $[^{188}\text{Re}]\text{ReO}_4^-$ was reduced to a $[^{188}\text{Re}]\text{Re}^\text{V}$-citrate precursor: an aqueous solution containing $[^{188}\text{Re}]\text{ReO}_4^-$ (~300-500 MBq), sodium citrate and stannous chloride ($\text{SnCl}_2$), at pH 5.5, was heated at 90 °C for 30 min, to give $[^{188}\text{Re}]\text{Re}^\text{V}$-citrate in > 95% yield, as previously described (20,21). Following this, $[^{188}\text{Re}]\text{Re}^\text{V}$-citrate (~130 – 210 MBq) was added to a pre-fabricated, lyophilized kit (Table S1), containing sodium carbonate, sodium tartrate, $\text{SnCl}_2$ and either DP1-PSMA or DP2-PSMA. The resulting radiolabeling solution (pH 8.0 – 8.5) was heated at 90 °C for 30 min, to form $[^{188}\text{Re}]\text{Re-DP1-PSMA}$ in 20-70% radiochemical yield and $[^{188}\text{Re}]\text{Re-DP2-PSMA}$ in 20-50% radiochemical yield (as determined by radio-HPLC): whilst formation of the desired products was reproducible, yields were not.

$[^{188}\text{Re}]\text{Re-DP1-PSMA}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMA}$ were subsequently isolated using reverse-phase HPLC, lyophilized and then reconstituted in PBS to yield the radiotracers in > 95% radiochemical purity. The purified $^{188}\text{Re}$ compounds were analyzed by reverse-phase radio-HPLC. Each $^{188}\text{Re}$ compound co-eluted with its non-radioactive $^{99m}\text{Tc}$ isotopolog and radioactive $^{99m}\text{Tc}$ analog (17), confirming not only the chemical identity of these new $^{188}\text{Re}$ agents, but also that they are isostructural and isoelectronic with their $^{99m}\text{Tc}$ analogs (Figure 1). Each Re and Tc compound consists of two closely eluting isomers, cis- and trans-$[\text{M}^\text{O}_2\text{(DPX-PSMA)}]_2^+$ (M = Re or Tc, $X = 1$ or 2) (17). The cis and trans designations denote the relative positions of the PSMAt moieties.

Solutions of each new $^{188}\text{Re}$ radiotracer were added to human serum and incubated at 37 °C for 24 h followed by analysis by reverse-phase analytical radio-HPLC, which demonstrated that $[^{188}\text{Re}]\text{Re-DP1-PSMA}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMA}$ are stable, with > 95% each radiotracer respectively observed intact in human serum over this timeframe (Figure S2).
Figure 1. A. Chemical structures of M-DP1-PSMA and M-DP2-PSMA, M = Tc (99mTc or 99Tc) and Re (natRe or 188Re). B. Reverse-phase C18-HPLC radiochromatograms showing co-elution of [188Re]Re-DP1-PSMA, [99mTc]Tc-DP1-PSMA and [natRe]Re-DP1-PSMA, and co-elution of [188Re]Re-DP2-PSMA, [99mTc]Tc-DP2-PSMA and [natRe]Re-DP2-PSMA.

Uptake of 99mTc and 188Re agents in prostate cancer cells

To assess the specificity of the new radiotracers for PSMA, [99mTc]Tc-DP1-PSMA and [99mTc]Tc-DP2-PSMA (100 kBq) were each incubated with DU145-PSMA+ prostate cancer cells (DU145 cells transfected to express PSMA receptor) (22). After 1 h incubation, uptake of each radiotracer was quantified. To assess specificity, each radiotracer was also (i) co-incubated with the PSMA-inhibitor, PMPA, with DU145-PSMA+ cells, and (ii) incubated with parental DU145 cells that do not express PSMA. [99mTc]Tc-DP1-PSMA and [99mTc]Tc-DP2-PSMA exhibited uptake in DU145-PSMA+ cells (12.4 ± 2.8 %AR [percentage added radioactivity], and 7.8 ± 1.3 %AR, respectively). This uptake was specific: DU145-PSMA+ cell uptake of [99mTc]Tc-DP1-PSMA and [99mTc]Tc-DP2-PSMA could be blocked with PMPA, and there was negligible uptake in parental DU145 cells (Figure 2). The uptake of [99mTc]Tc-DP1-PSMA and [99mTc]Tc-DP2-PSMA was also studied in LNCaP prostate cancer cells, which natively express PSMA, and PC3 cells which, like parental DU145 cells, do not express PSMA. In LNCaP cells, uptake of [99mTc]Tc-DP1-PSMA and [99mTc]Tc-DP2-PSMA measured 3.7 ± 1.2 %AR and 3.0 ± 0.8 %AR, respectively, whilst uptake of both radiotracers in PC3 cells measured less than 0.3 %AR. Uptake in LNCaP cells could also be blocked with PMPA.

The cellular localization of [99mTc]Tc-DP1-PSMA and [99mTc]Tc-DP2-PSMA was also evaluated in DU145-PSMA+ and LNCaP cells over time (Figure 2). Uptake of both radiotracers increased over 2 h, and the majority of the cell-associated radioactivity was found in the internalized cell fraction at all measured time points for both PSMA-expressing cell lines. [99mTc]Tc-DP1-PSMA uptake (both surface-bound and internalized radioactivity) was slightly higher than that of [99mTc]Tc-DP2-PSMA.
Additionally, the uptake of $^{188}\text{Re}$DP1-PSMA and $^{188}\text{Re}$DP2-PSMA (100 kBq) was also assessed in the abovementioned prostate cancer cell lines: both radiotracers exhibited uptake in DU145-PSMA+ cells (13.5 ± 2.7%AR and 8.3 ± 0.7 %AR, respectively) and LNCaP cells (2.8 ± 1.6%AR and 2.6 ± 1.6 %AR, respectively). This uptake was also specific: uptake of $^{188}\text{Re}$DP1-PSMA and $^{188}\text{Re}$DP2-PSMA could be blocked with PMPA, and there was negligible uptake in parental DU145 cells and PC3 cells (Figure 2).

Figure 2. A. Uptake of radiotracers in PSMA-positive and PSMA-negative prostate cancer cells. B. Time course uptake and localization of $^{99m}\text{Tc}$ radiotracers in DU145-PSMA cells and LNCaP cells. Data are presented as mean ± SD, $n = 3-4$ biological repeats performed in triplicate.
The biodistributions of $[^{99m}Tc]$Tc-DP1-PSMAT and $[^{99m}Tc]$Tc-DP2-PSMAT were assessed in male SCID/Beige mice bearing DU145-PSMA+ xenograft tumors (Figure 3). Each mouse was administered either $[^{99m}Tc]$Tc-DP1-PSMAT or $[^{99m}Tc]$Tc-DP2-PSMAT, and euthanized at 2 h post-injection ($n = 5$), followed by organ harvesting for ex vivo radioactivity counting. High amounts of each tracer were observed in tumors 2 h post-injection; for $[^{99m}Tc]$Tc-DP1-PSMAT, $[^{99m}Tc]$Tc concentration measured 18.0±3.5 %ID/g (percentage injected dose per gram) and for $[^{99m}Tc]$Tc-DP2-PSMAT, $[^{99m}Tc]$Tc concentration measured 29.4±6.3 %ID/g.

To assess specificity of each radiotracer, separate groups of animals, also bearing DU145-PSMA+ tumors, were co-administered either $[^{99m}Tc]$Tc-DP1-PSMAT and PMPA, or $[^{99m}Tc]$Tc-DP2-PSMAT and PMPA, to inhibit PSMA-mediated uptake of radiotracer ($n = 5$). In mice bearing DU145-PSMA+ tumors, co-administration of PMPA substantially decreased uptake of both $[^{99m}Tc]$Tc-DP1-PSMAT and $[^{99m}Tc]$Tc-DP2-PSMAT in tumors. For $[^{99m}Tc]$Tc-DP1-PSMAT, co-administration decreased uptake to 0.91±0.29 %ID/g in the tumor (compared to administration of $[^{99m}Tc]$Tc-DP1-PSMAT only: mean difference = 17.12 %ID/g, $p = 4 \times 10^{-4}$). For $[^{99m}Tc]$Tc-DP2-PSMAT, co-administration decreased uptake to 0.76±0.45 %ID/g in the tumor (compared to administration of $[^{99m}Tc]$Tc-DP2-PSMAT only: mean difference = 28.62 %ID/g, $p = 5 \times 10^{-4}$).

For both radiotracers, the concentration of $[^{99m}Tc]$Tc radioactivity in kidneys 2 h post-injection was high (Figure 3). Higher amounts of $[^{99m}Tc]$Tc-DP2-PSMAT were measured in kidneys 2 h post-injection (183.3±23.8 %ID/g) compared to $[^{99m}Tc]$Tc-DP1-PSMAT (115.9±27.3 %ID/g, mean difference = 67.4 %ID/g, $p = 0.003$). Notably, for animals administered $[^{99m}Tc]$Tc-DP1-PSMAT, co-administration of PMPA significantly decreased retention of $[^{99m}Tc]$Tc radioactivity in kidneys. In contrast, although co-administration of PMPA also decreased radioactivity concentration in the kidneys for animals injected with $[^{99m}Tc]$Tc-DP2-PSMAT, this effect was much less pronounced. There were also significant amounts of both radiotracers ($[^{99m}Tc]$Tc-DP1-PSMAT: 12.0±5.3 %ID/g; $[^{99m}Tc]$Tc-DP2-PSMAT: 7.7±3.4 %ID/g) that residualized in the spleen, which is known to express low levels of PSMA and accumulate PSMA-targeted radiotracers (5,6,9). As expected, co-administration of PMPA significantly decreased retention of $[^{99m}Tc]$Tc radioactivity in the spleen for both radiotracers ($[^{99m}Tc]$Tc-DP1-PSMAT: 0.2±0.06 %ID/g, mean difference = 11.8 %ID/g, $p = 0.008$; $[^{99m}Tc]$Tc-DP2-PSMAT: 0.3±0.18 %ID/g, mean difference = 7.4 %ID/g, $p = 0.008$).

Additionally, groups of mice bearing non-PSMA-expressing parental DU145 tumors were also administered these $[^{99m}Tc]$Tc radiotracers. For these groups, tumor uptake of $[^{99m}Tc]$Tc-DP1-PSMAT decreased to 0.24±0.07 %ID/g, and tumor uptake of $[^{99m}Tc]$Tc-DP2-PSMAT decreased to 0.18±0.07 %ID/g (Figure S3).

In SPECT/CT scans of animals administered either $[^{99m}Tc]$Tc-DP1-PSMAT or $[^{99m}Tc]$Tc-DP2-PSMAT only, tumors could be clearly delineated at both 2 h (Figure 3) and 24 h post-injection (Figure S4). The kidneys and bladder were also clearly visible across these timepoints, consistent with ex vivo biodistribution data. SPECT/CT also showed negligible tumor uptake for animals either co-administered PMPA or animals bearing DU145 tumors that do not express PSMA receptor (Figure 3). For all animals administered either $[^{99m}Tc]$Tc-DP1-PSMAT only or $[^{99m}Tc]$Tc-DP2-PSMAT only, the spleen was also identified in SPECT/CT scans acquired at 2 h post-injection. Co-administration of PMPA decreased spleen uptake of both radiotracers.
Figure 3. A. Biodistribution (2 h post-injection) of SCID/Beige mice bearing DU145-PSMA+ prostate cancer tumors, administered either $^{99m}$Tc or $^{188}$Re radiotracers intravenously. To assess specificity of radiotracer uptake, additional groups of mice were also co-administered radiotracer and PMPA. See also Tables S3 and S4, Supplemental Data. B. Whole body SPECT/CT maximum intensity projections of SCID/Beige mice bearing either DU145-PSMA+ tumors or DU145 tumors, administered either $^{99m}$TcDP1-PSMA (left) or $^{99m}$Tc-DP2-PSMA (right), 2 h post-injection. To inhibit uptake in DU145-PSMA+ tumors, animals were also co-administered PMPA.
The biodistributions of the $^{188}$Re radiotracers, $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$, were first assessed in SCID/Beige mice bearing DU145-PSMA+ tumors (Figure 3). Radioactivity concentration in the tumors of animals administered $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ measured 27.7±6.4 %ID/g at 2 h post-injection, whilst that of animals given $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$ measured 19.2±8.6 %ID/g. Both compounds cleared circulation via a renal pathway, as evidenced by high concentrations of radioactivity measured in the kidneys ($[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ measured 88.3±18.8 %ID/g, $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$ measured 96.8±16.3 %ID/g). Biodistribution data also indicated that both compounds had low retention in non-target, healthy organs/tissues, except for the spleen. In this experiment, there were no notable significant differences between the biodistribution profiles of $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$ at 2 h post-injection. As expected, co-administration of PMPA significantly inhibited uptake of both $^{188}$Re radiotracers in the tumor and spleen.

Urine was collected from mice administered either $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ or $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$ at 2 h post-injection and analyzed by reverse-phase radio-HPLC. Radio-chromatograms showed that both $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$ were highly stable and were cleared from the blood pool and excreted chemically intact (Figure S5).

With a view to developing a molecular $[^{188}\text{Re}]\text{Re}$-labeled agent for PSMA PRRT, we further characterized the biodistribution profiles of $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$ in male athymic nude mice bearing LNCaP xenografts (Figure 4), which recapitulate clinical metastatic prostate cancer more closely than DU145-PSMA+ xenografts. Importantly, the $^{188}$Re agents exhibited significant retention in tumors up to at least one day post-administration. In mice administered $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$, LNCaP tumor uptake measured 7.0±2.3 %ID/g at 2 h post-injection and 2.9±0.8 %ID/g at 24 h post-injection. For $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$, LNCaP tumor uptake measured 9.8±2.8 %ID/g at 2 h post-injection and 7.6±4.4 %ID/g at 24 h post-injection. With the exception of the kidneys, radioactivity concentrations in other organs were similar to that observed in prior experiments with male SCID/Beige mice.

The concentration of $^{188}$Re agents in kidneys was relatively high: $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ in kidneys measured 137.1±34.6 %ID/g and that of $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$ measured 162.8±31.8 %ID/g at 2 h post-injection. These decreased to 29.4±4.9 %ID/g and 37.0±12.2 %ID/g, respectively at 24 h post-injection. Separate groups of animals were co-administered the plasma expander gelofusin, which has previously been used to reduced kidney retention of PRRT agents and minimize potential nephrotoxicity. For animals co-administered $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ and gelofusin, kidney retention decreased to 108.8±13.3 %ID/g at 2 h post-injection ($p = 0.11$); for $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$, kidney retention decreased to 127.6±17.7 %ID/g ($p = 0.046$).

Lastly, to further assess biological equivalence of pairs of $^{99m}\text{Tc}$ and $^{188}$Re agents, the biodistributions of $[^{99m}\text{Tc}]\text{Tc-DP1-PSMAt}$ and $[^{99m}\text{Tc}]\text{Tc-DP2-PSMAt}$ were also assessed in athymic nude mice bearing LNCaP prostate cancer tumors. Importantly, the biodistribution patterns and clearance pathways of $[^{99m}\text{Tc}]\text{Tc-DP1-PSMAt}$ and $[^{188}\text{Re}]\text{Re-DP1-PSMAt}$ were highly similar when compared in the same mouse model (either male athymic nude mice bearing LNCaP xenografts or male SCID/Beige mice bearing DU145-PSMA+ xenograft tumors). The same near-equivalent biodistribution patterns were observed for $[^{99m}\text{Tc}]\text{Tc-DP2-PSMAt}$ and $[^{188}\text{Re}]\text{Re-DP2-PSMAt}$. Between analogous $^{99m}\text{Tc}$ and $^{188}$Re radiotracers, the most notable difference in biodistribution behavior was that $^{99m}\text{Tc}$ radiotracers demonstrated consistently higher kidney residualization compared to that of $^{188}$Re radiotracers.
Figure 4. Biodistribution of $^{188}$Re in athymic nude mice bearing LNCaP prostate cancer tumors, administered either $^{188}$Re-DP1-PSMAt or $^{188}$Re-DP2-PSMAt A at 2 h post-injection, B at 24 h post-injection, and C at 2 h post-injection with co-administration of gelofusin. Additionally the biodistributions of D $^{99m}$Tc-DP1-PSMAt and $^{99m}$Tc-DP2-PSMAt at 2 h post-injection were measured. See also Tables S5-S8, Supplemental Data.

Discussion

Theranostic PSMA-targeted radiopharmaceuticals have had extraordinary impact in prostate cancer care in healthcare settings where they are available. We have developed two pairs of chemically analogous theranostic agents based on the generator-produced radionuclides, $^{99m}$Tc and $^{188}$Re. Radio-HPLC alongside careful chemical characterization of non-radioactive/long-lived isotopologs (17) demonstrates that Tc and Re pairs are chemical analogs and isostructural. Consequently, each pair exhibits highly similar biological behavior in in vitro and in vivo models of prostate cancer. Importantly, both $^{188}$Re and $^{99m}$Tc-labeled complexes of DP1-PSMAt and DP2-PSMAt show high accumulation in PSMA-expressing tumors and prostate cancer cells, and in vivo, rapidly clear circulation via a renal pathway, with minimal retention in healthy tissues. Furthermore, in vivo, these four radiotracers all demonstrate significant retention in prostate cancer tumors up to 24 h post-injection.

In a clinical context, the favorable and near-identical biological behaviors of chemically analogous $^{99m}$Tc and $^{188}$Re radiotracers bring about the possibility of using $^{99m}$Tc molecular imaging to predict the biodistribution, accumulation and dosimetry of a complementary $^{188}$Re PRRT agent. Our novel theranostic pairs, M-DP1-PSMAt and M-DP2-PSMAt (M = $^{99m}$Tc, $^{188}$Re), which utilize economical generator-produced isotopes, have strong potential utility for this purpose in prostate cancer treatment, particularly in LMICs. Additionally, the DP chemical platform underpinning these theranostic radiotracers is an excellent candidate.
for development of other peptide-based radiotracers: this technology could increase clinical use of receptor-targeted $^{99m}$Tc and $^{188}$Re radiopharmaceuticals, and widen patient access to the benefits of molecular theranostic agents.

In this work, $[^{99m}$Tc]Tc-DP1-PSMA$t$, $[^{99m}$Tc]Tc-DP2-PSMA$t$, $[^{188}$Re]Re-DP1-PSMA$t$ and $[^{188}$Re]Re-DP2-PSMA$t$ are all subject to post-synthetic HPLC purification and isolation procedures. Using HPLC, all these compounds can be easily separated from unreacted ligand as well as unreacted $^{99m}$Tc/$^{188}$Re-labeled precursors, yielding radiotracers of extremely high specific activity; i.e. with the exception of decay products, there is minimal unlabeled DP1-PSMA or DP2-PSMA present in final radiotracer formulations. This culminates in extremely high accumulation in PSMA-expressing prostate cancer cells in vitro and tissue in vivo (tumors, spleen, and in the case of DP1-PSMA derivatives, the kidneys).

In some in vivo experiments, there were statistically significant differences in tumor uptake between DP1 and DP2 bioconjugate derivatives, however, these differences were not recapitulated in alternative experiments. For example, in experiments using SCID/Beige mice bearing DU145-PSMA+ tumors at 2 h post-injection, there was significantly higher $[^{99m}$Tc]Tc-DP2-PSMA$t$ in tumors (29.4±6.3 %ID/g) compared to $[^{99m}$Tc]Tc-DP1-PSMA$t$ (18.0±3.5 %ID/g, mean difference = 11.4 %ID/g, $p = 0.01$). However, in experiments using athymic nude mice bearing LNCaP tumors, no statistically significant difference in tumor uptake of these two $^{99m}$Tc radiotracers was observed at 2 h post-injection. Between homologous DP1 and DP2 radiotracers, the most notable difference is that M-DP1-PSMA (M = $[^{99m}$Tc]Tc or $[^{188}$Re]Re) exhibits significantly higher spleen retention compared to M-DP2-PSMA at 2 h post-injection (for comparisons of $[^{99m}$Tc]Tc DP1-PSMA$t$ vs $[^{99m}$Tc]Tc DP2-PSMA$t$, or $[^{188}$Re]Re-DP1-PSMA$t$ vs $[^{188}$Re]Re-DP2-PSMA$t$, in SCID/beige or athymic nude mice, $p \leq 0.05$).

All four radiotracers exhibit high kidney residualization. In the case of DP1-PSMA derivatives, it is likely that this retention is in part mediated by PSMA (in SCID/beige mice, $[^{99m}$Tc]Tc-DP1-PSMA: 116 ± 27 %ID/g, with PMPA: 38 ± 2.9 %ID/g, $p = 2.88 \times 10^{-3}$; $[^{188}$Re]Re-DP1-PSMA: 88 ± 19 %ID/g, with PMPA: 38 ± 3.4 %ID/g, $p = 1.16 \times 10^{-3}$). Murine kidney tissues, specifically proximal renal tubules and Bowman’s capsule, express PSMA (23). DP2 radiotracers consistently demonstrated higher kidney retention than DP1 radiotracers. We attribute this to the comparatively higher lipophilicity of DP2 derivatives (17). Prior comparative in vivo murine studies have observed that increases in the lipophilicity of PSMA$^*$-derived compounds increases their kidney retention (24). Whilst co-administration of gelofusin decreased kidney retention of $^{188}$Re agents at 2 h post-administration, this observed decrease was not statistically significant. In light of the comparatively similar tumor uptake of DP1 and DP2 radiotracers, but the higher kidney retention of DP2 radiotracers, we postulate that DP1-based radiotracers are better clinical theranostic candidates.

Compared to the existing $[^{99m}$Tc- and $^{188}$Re-radiolabeled tracers, $[^{99m}$Tc]Tc-MIP-1404 (5), $[^{99m}$Tc]Tc-PSMA-I&S (6), and $[^{99m}$Tc]Tc-EDDA/HYNIC-iPSMA (9), and $[^{188}$Re]Re-PSMA-GCK01 (16), respectively, the new DP-based radiotracers demonstrate either decreased or comparable residualization in murine liver, and either increased or comparable blood clearance, at 1 – 2 h post-administration (Table S2).

Like existing PSMA-targeted $^{99m}$Tc radiotracers, both $[^{99m}$Tc]Tc-DP1-PSMA$t$ and $[^{99m}$Tc]Tc-DP2-PSMA$t$ can be formulated using a kit: our existing protocols and prototype kit radiosynthetic methods enable high radiochemical yields of desired radiotracer (80 – 90%) by heating a saline solution of generator-produced $[^{99m}$Tc]TcO$_4^-$ with kit components at 100 °C for 5 min (17–19). We elected to use a two-step protocol to prepare $^{188}$Re-radiolabeled agents, in which a $[^{188}$Re]Re$^{3+}$-citrate precursor is reacted with either DP1-PSMA$t$ or DP2-PSMA$t$ to yield the desired $^{188}$Re radiotracers. This procedure is similar to prior radiosyntheses of $[^{188}$Re]Re[ReO$_4$]$^{3+}$-labeled phosphate-containing P$_2$S$_2$ and P$_2$N$_2$ tetradentate chelator derivatives (20,21). However, complexes of the latter chelators were obtained in > 90% radiochemical yield; the highest radiochemical yields obtained for $[^{188}$Re]Re-DP1-PSMA$t$ and $[^{188}$Re]Re-DP2-PSMA$t$ were ~70% and ~50% respectively. We also note that the $[^{188}$Re][ReO$_4$]$^{3+}$-based complex, $[^{188}$Re]Re-PSMA-GCK01, can be obtained in 78% radiochemical yield and 96% radiochemical purity, using a one-pot radiochemical procedure (16). This procedure involves heating an aqueous solution of precursor GCK01, $[^{188}$Re]ReO$_4^-$, citrate and ascorbic acid at pH 2.0 – 3.5 at high temperature for 1 hour, followed by neutralization, further heating for 5 min, and final C18 Sep-Pak purification. We are currently optimizing kit-based formulations to increase the radiochemical
yields of DP-based $^{99m}$Tc and $^{188}$Re radiotracers, and obviate the requirement for post-synthetic purification procedures to remove unreacted $[^{99m}$Tc]$Tc$ and $[^{188}$Re]$Re$ precursors.

Conclusion

We have developed new PSMA-targeting $^{99m}$Tc/$^{188}$Re theranostic agents, using versatile diphosphine chemical platforms. These novel radiotracers can be prepared using eluate from bench-top $^{99m}$Tc and $^{188}$Re generators and chemical kits. The resulting isostructural $^{99m}$Tc and $^{188}$Re pairs show near-equivalent biological behaviors in models of prostate cancer. We are further developing optimized kit-based formulations to enable near-quantitative radiochemical yields to obviate purification steps post-radiolabeling. Our new, generator-based theranostic agents have potential to provide access to the benefits of PSMA-targeted diagnostic imaging and systemic radiotherapy in healthcare settings that do not routinely have access to either reactor-produced $^{177}$Lu radiopharmaceuticals or PET/CT infrastructure.

Disclosure

The authors have submitted a patent application describing the intellectual property described herein. No other potential conflicts of interest relevant to this article exist.

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References


KEY POINTS

QUESTION: Are molecular theranostic radiotracers based on technetium-99m and rhenium-188 feasible for diagnostic imaging and systemic radiotherapy of prostate cancer?

PERTINENT FINDINGS: Pairs of chemically analogous $^{99m}$Tc/$^{188}$Re radiotracers show equivalent uptake in PSMA-expressing prostate cancer cells, and favorable, highly similar biodistribution profiles in a mouse model of prostate cancer.

IMPLICATIONS FOR PATIENT CARE: These generator-based theranostic agents have potential to provide access to the benefits of PSMA-targeted diagnostic imaging and systemic radiotherapy in healthcare settings that do not routinely have access to either cyclotron- or reactor-produced radionuclides.

GRAPHICAL ABSTRACT