

## Alpha-melanocyte stimulating hormone peptide-targeted melanoma imaging

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## 1. ABSTRACT

Melanocortin-1 (MC1) receptor is an attractive melanoma-specific target over-expressed on the surface of melanoma cells. Alpha-melanocyte stimulating hormone (alpha-MSH) peptide analogues can specifically bind MC1 receptors with nanomolar binding affinities, making alpha-MSH peptide analogues very promising candidates for developing effective melanoma-specific imaging probes. This review highlights the strategies being used to design alpha-MSH peptide radiopharmaceuticals to target MC1 receptors and some recent developments of radiolabeled alpha-MSH peptide analogues for melanoma imaging.

## 2. INTRODUCTION

Malignant melanoma is the most lethal form of skin cancer and is sixth most commonly diagnosed cancer with an increasing incidence in the United States. It is predicted that 59,940 new cases will be diagnosed and 8,110 fatalities will occur in the year 2007 (1). At the

present time, more than 1.3% of Americans will develop malignant melanoma during their lifetime (2). Among young adults, melanoma is the most commonly diagnosed malignancy (3). Early diagnosis and prompt surgical removal of primary melanoma lesions are patients' best hope for cures. Melanoma metastases are very aggressive and the survival time for patients with metastatic melanoma averages 3-15 months (4). Unfortunately, there is no satisfactory treatment for metastatic melanoma due to its resistance to current chemotherapy and immunotherapy regimens (5). Early diagnosis with accurate staging of malignant melanoma is critical for appropriate treatment decisions and may provide the melanoma patients the best opportunities for cures or prolonged survival (6-8).

The basis for current clinical diagnosis of melanoma is built on the morphologies of the melanoma tumor, which includes asymmetry, border irregularity, color variegation and diameter bigger than 6 mm. However,

the diagnostic accuracy of melanoma is only about 65% (9). Conventional non-invasive imaging techniques, such as chest radiography, ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI), are commonly used for identifying patients with melanoma metastases in lung and brain area (10-13). However, the conventional non-invasive imaging techniques have limited sensitivity (57%-81%) and specificity (45-87%) for the detection of single melanoma lesions due to lack of soft tissue contrast and/or melanoma specificity (14-19). 2-[ $^{18}\text{F}$ ]fluoro-2-deoxy-D-glucose ([ $^{18}\text{F}$ ]FDG) PET is the most commonly used radioimaging agent in the diagnosis and staging of cancer including melanoma. The increased accumulation of [ $^{18}\text{F}$ ]FDG in melanoma cells is due to a higher metabolic rate than normal cells (20). Hence, [ $^{18}\text{F}$ ]FDG is not a melanoma-specific imaging agent. [ $^{18}\text{F}$ ]FDG PET imaging is more accurate than conventional imaging techniques for melanoma detection. [ $^{18}\text{F}$ ]FDG PET imaging exhibited 100% detection rate for metastatic melanoma greater than 10 mm (21). However, [ $^{18}\text{F}$ ]FDG PET imaging only detected 23% melanoma metastases smaller than 5 mm (21). Moreover, it has been reported that some melanoma cells were undetectable by [ $^{18}\text{F}$ ]FDG since they used substrates other than glucose as energy sources (22).

Currently, melanocortin-1 (MC1) receptor is a very promising melanoma-specific target for the development of effective imaging probes. MC1 receptor is over-expressed in both melanotic and amelanotic melanomas (23-25). The melanocortin receptors belong to the superfamily of G-protein coupled receptors (GPCRs). At the present time, five melanocortin receptors, namely MC1 to MC5 receptors, have been identified and cloned (26-32). The MC1 receptor is expressed in melanocytes and leukocytes and is mainly involved in skin pigmentation and animal coat coloration (26, 33). It has been demonstrated that the MC1 receptors are over-expressed on the surfaces of human and mouse melanoma cells (34-36). More than 80% of human metastatic melanoma tumor samples have been found to display MC1 receptors (23). Radiolabeled alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) peptide analogues exhibit nanomolar MC1 receptor binding affinities, making them very promising melanoma-specific imaging probes for melanoma detection. This review highlights the recent developments of radiolabeled MC1 receptor-targeting  $\alpha$ -MSH peptides as imaging probes for melanoma imaging. Two types of radiolabeled  $\alpha$ -MSH peptides, namely linear and metal-cyclized peptides, will be the focus of this review. The results highlighted in this review demonstrate the rationales for developing new MC1 receptor-targeting  $\alpha$ -MSH peptide radiopharmaceuticals as melanoma-specific imaging probes for melanoma detection.

### 3. RADIOLABELED LINEAR ALPHA-MSH PEPTIDE ANALOGUES FOR MELANOMA IMAGING

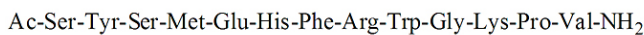
Wild type  $\alpha$ -MSH is a linear tridecapeptide (Ac-Ser<sup>1</sup>-Tyr<sup>2</sup>-Ser<sup>3</sup>-Met<sup>4</sup>-Glu<sup>5</sup>-His<sup>6</sup>-Phe<sup>7</sup>-Arg<sup>8</sup>-Trp<sup>9</sup>-Cys<sup>10</sup>-Lys<sup>11</sup>-Pro<sup>12</sup>-Val<sup>13</sup>-NH<sub>2</sub>, Figure 1), which is involved in the control of skin pigmentation. The biological activity of  $\alpha$ -

MSH is mediated through interactions with the MC1 receptor (37). MC1 receptor agonists are internalized into the melanoma cells upon binding to MC1 receptors (38, 39). During the 1970s to 1980s, intensive research efforts were focused on the development of tritium-labeled linear  $\alpha$ -MSH analogues (40-44). [ $^3\text{H}$ ]-Nva<sup>1,4,13</sup>, D-Phe<sup>7</sup>]- $\alpha$ -MSH was reported to be suitable for tissue distribution and stability studies *in vivo* (44). However, the tritium-labeled linear  $\alpha$ -MSH analogues suffered with relatively low specific activity (12.21 GBq/ $\mu\text{mol}$ ), making *in vivo* tumor targeting inefficient (25). The specific activity was dramatically increased by 10 times through labeling  $\alpha$ -MSH analogues with  $^{125}\text{I}$  on Tyr<sup>2</sup> (25). The preparation and application of  $^{125}\text{I}$ - $\alpha$ -MSH,  $^{125}\text{I}$ -[Nle<sup>4</sup>]- $\alpha$ -MSH and  $^{125}\text{I}$ -[Nle<sup>4</sup>, D-Phe<sup>7</sup>]- $\alpha$ -MSH in receptor binding assays were reported in the literatures (34, 44-49). Currently, the most widely used linear  $\alpha$ -MSH peptide analogue is  $^{125}\text{I}$ -[Nle<sup>4</sup>, D-Phe<sup>7</sup>]- $\alpha$ -MSH (NDP-MSH) analogue (Figure 1), which is referred to as a “gold” standard due to its sub-nanomolar receptor binding affinity (39). However, the utilization of  $^{125}\text{I}$ -NDP-MSH is mainly limited in *in vitro* studies due to the dehalogenation reaction of  $^{125}\text{I}$ -NDP-MSH *in vivo* (44). Hence, several halogen labeling approaches were developed to reduce the *in vivo* dehalogenation. It was reported that NDP-MSH radiolabeled with *N*-succinimidyl 3-iodobenzoate (SIB) or *N*-succinimidyl 4-iodobenzoate (PIB) was inert to the *in vivo* dehalogenation (50, 51). Labeling of NDP-MSH at Lys<sup>11</sup> with *N*-succinimidyl 3- [ $^{125}\text{I}$ ]iodobenzoate, yielded [Nle<sup>4</sup>, D-Phe<sup>7</sup>, Lys<sup>11</sup>-( $^{125}\text{I}$ )IBA]- $\alpha$ -MSH ( $^{125}\text{I}$ -IBA-NDP-MSH), which exhibited higher receptor binding affinity than  $^{131}\text{I}$ -NDP-MSH (51). Reduced radioactivity in the thyroid and stomach of mice, intravenously injected with  $^{125}\text{I}$ -IBA-NDP-MSH compared to  $^{131}\text{I}$ -NDP-MSH, demonstrated its resistance to the *in vivo* dehalogenation. Likewise, *N*-succinimidyl 4- [ $^{18}\text{F}$ ]fluorobenzoate labeled NDP-MSH ( $^{18}\text{F}$ -PFB-NDP-MSH) exhibited rapid clearance with little evidence for defluorination in normal mice (52). However, no biodistribution of  $^{125}\text{I}$ -IBA-NDP-MSH and  $^{18}\text{F}$ -PFB-NDP-MSH have been reported in melanoma-bearing mice.

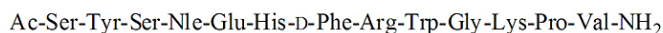
Another method to radiolabel linear  $\alpha$ -MSH peptide analogues is through the conjugation of a bifunctional radionuclide chelator. Bifunctional chelators such as Ac-Cys-Gly-Cys-Gly- (CGCG), tetrafluorophenyl mercapto-acetylglucylglycyl-gamma-aminobutyrate (MAG<sub>2</sub>), diethylenetriamine pentaacetate (DTPA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) are usually attached to the N-terminus of the  $\alpha$ -MSH peptides. CGCG and MAG<sub>2</sub> are N<sub>2</sub>S<sub>2</sub> and N<sub>3</sub>S chelation systems that are able to easily coordinate  $^{99\text{m}}\text{Tc}$  or  $^{188}\text{Re}$  (53, 54). It was reported that  $^{99\text{m}}\text{Tc}$ -CGCG-NDP-MSH (Figure 1) exhibited greater tumor to blood and tumor to muscle ratios than  $^{99\text{m}}\text{Tc}$ -MAG<sub>2</sub>-NDP-MSH and  $^{125}\text{I}$ -Tyr<sup>2</sup>-NDP-MSH at 30 min, 1 and 4 h post-injection (54). However, the moderate melanoma uptake of  $^{99\text{m}}\text{Tc}$ -CGCG-NDP-MSH (6.52 $\pm$ 1.11 %ID/g at 30 min post-injection) prevented its further evaluation. DTPA and DOTA are good metal chelators and are able to form stable complexes with a variety of radiometals. Indium-111-labeled DTPA-bis-NDP was reported to be able to image melanoma with 89% sensitivity in a limited phase I clinical trial (55).

## Peptide radiopharmaceuticals for melanoma imaging

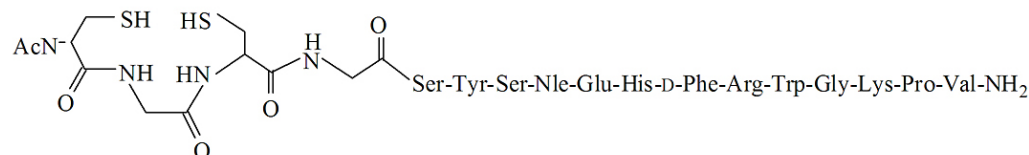
$\alpha$ -MSH:



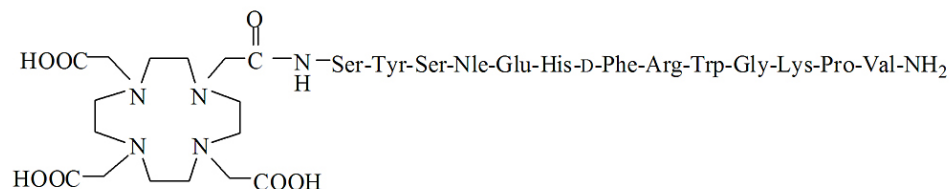
[Nle<sup>4</sup>, D-Phe<sup>7</sup>] $\alpha$ -MSH (NDP-MSH):



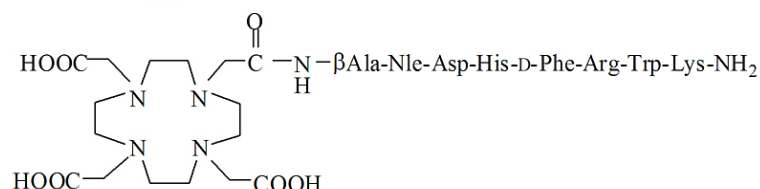
CGCG-NDP-MSH:



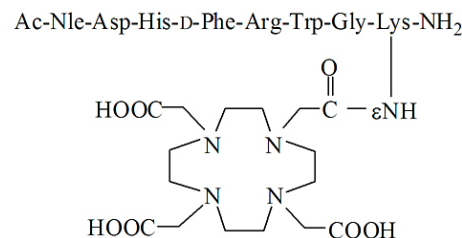
DOTA-NDP-MSH:



DOTA-MSH<sub>OCT</sub>:



DOTA-NAPamide:



**Figure 1.** Schematic structures of linear  $\alpha$ -MSH peptide analogues.

However, high renal and liver uptake compromised its imaging capabilities in those organs. Recently,  $^{111}\text{In}$ -labeled short linear DOTA-conjugated NDP analogues, such as  $^{111}\text{In}$ -DOTA-NDP-MSH and  $^{111}\text{In}$ -DOTA-MSH<sub>OCT</sub> (Figure 1), were reported as potential melanoma imaging agents (24, 56).  $^{111}\text{In}$ -DOTA-MSH<sub>OCT</sub> exhibited  $4.31 \pm 0.30$  %ID/g and  $1.17 \pm 0.13$  %ID/g at 4 and 24 h post-injection in B16/F1 melanoma-bearing mice. Although  $^{111}\text{In}$ -DOTA-

MSH<sub>OCT</sub> exhibited significant ( $p < 0.05$ ) lower tumor uptake than  $^{111}\text{In}$ -DOTA-NDP-MSH at 4, 24 and 48 h post-injection, the accumulation of  $^{111}\text{In}$ -DOTA-MSH<sub>OCT</sub> activity in normal organs was lower than that of  $^{111}\text{In}$ -DOTA-NDP-MSH at 4, 24 and 48 h post-injection except for the kidneys (24). The autoradiographs of tissue section with metastatic melanoma showed that  $^{111}\text{In}$ -DOTA-MSH<sub>OCT</sub> was taken up by both melanotic and amelanotic

melanoma metastases (24). More recently,  $^{111}\text{In}$ -DOTA-NAPamide (Figure 1) was reported to exhibit a more favorable biodistribution profile (higher tumor uptake and less renal uptake) than  $^{111}\text{In}$ -DOTA-MSH<sub>OCT</sub> in the same melanoma mouse model (57).  $^{111}\text{In}$ -DOTA-NAPamide displayed the tumor uptake of  $7.56 \pm 0.51$  %ID/g and the kidney uptake of  $5.06 \pm 0.32$  %ID/g at 4 h post-injection. Compared to the  $^{111}\text{In}$ -DOTA-MSH<sub>OCT</sub>, DOTA was coupled to the side chain of Lys on the C-terminus rather than N-terminus in  $^{111}\text{In}$ -DOTA-NAPamide. The amino group at the N-terminus was acetylated and the Gly<sup>10</sup> was introduced in the peptide of  $^{111}\text{In}$ -DOTA-NAPamide. All these changes resulted in the improvement of the tumor to kidney uptake ratio of the  $^{111}\text{In}$ -DOTA-NAPamide compared to the  $^{111}\text{In}$ -DOTA-MSH<sub>OCT</sub>. The substitution of  $^{111}\text{In}$  with  $^{67}\text{Ga}$  further improved the tumor to kidney ratio of  $^{67}\text{Ga}$ -DOTA-NAPamide (57). The autoradiographs of tissue section with metastatic melanoma showed that  $^{67}\text{Ga}$ -DOTA-NAPamide was taken up by both melanotic and amelanotic melanoma metastases, highlighting the suitability of  $^{67}\text{Ga}$ -DOTA-NAPamide for melanoma metastases imaging. Melanoma lesions were visualized with  $^{68}\text{Ga}$ -DOTA-NAPamide in preclinical PET studies (57), demonstrating the potential of  $^{68}\text{Ga}$ -DOTA-NAPamide as a PET imaging probe for melanoma detection.

#### 4. RADIOLABELED METAL-CYCLIZED ALPHA-MSH PEPTIDE ANALOGUES FOR MELANOMA IMAGING

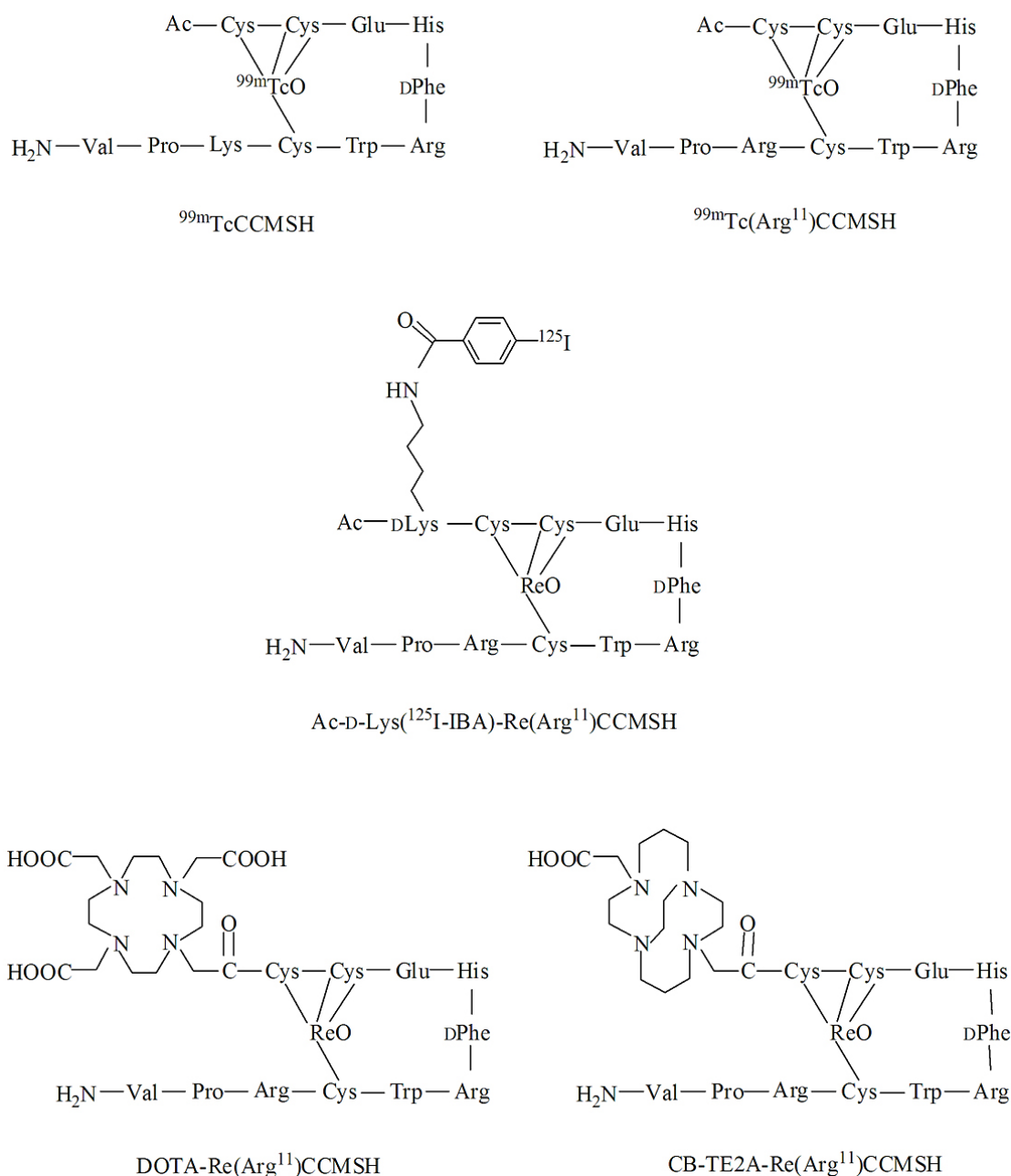
Radiolabeled metal-cyclized  $\alpha$ -MSH peptide analogues are another class of peptides with higher melanoma tumor uptake and superior tumor retention. Peptide cyclization was used to improve the *in vivo* stability and binding affinity of the peptide (58-60). Cyclic peptides possess less conformational freedom than linear peptides due to the stabilization of secondary structures such as beta turns, making cyclic peptides better fit receptor binding pocket which enhances binding affinities. A novel class of metal-cyclized  $\alpha$ -MSH peptide analogues was developed for melanoma imaging and therapy over the past several years (39, 56, 61-71). The cyclic  $\alpha$ -MSH peptide analogues incorporated non-radioactive or radioactive metals into their structures while retaining high affinities for MC1 receptors. Cyclization and radiolabeling of the peptide could be simultaneously achieved during the radiolabeling process. The metal cyclization made the peptide resistant to chemical and proteolytic degradation *in vivo* (39, 61). Initially, the  $\alpha$ -MSH peptide analogue (Cys<sup>4,10</sup>, D-Phe<sup>7</sup>)- $\alpha$ -MSH<sub>4-13</sub> (APOMSH) was synthesized and cyclized with non-radioactive rhenium (61). However, the rhenium cyclization dramatically decreased the binding affinity of ReO-APOMSH ( $K_i=66$  nM). Hence, another Cys was introduced to the N-terminus of the peptide sequence to yield (Cys<sup>3,4,10</sup>, D-Phe<sup>7</sup>)- $\alpha$ -MSH<sub>3-13</sub> (CCMSH). The inclusion of another Cys drove the metal-coordination sphere away from the His-D-Phe-Arg-Trp core receptor binding sequence by taking advantage of the higher binding affinity of sulfur than nitrogen for Tc and Re coordination. The binding affinity of ReO-CCMSH was increased to nanomolar ( $K_i=2.9$  nM). The radioactive congener of

ReCCMSH,  $^{99\text{m}}\text{Tc}$ -CCMSH, (Figure 2) exhibited rapid high tumor uptake and fast whole-body clearance in B16/F1 melanoma-bearing mice. The tumor uptake was  $10.74 \pm 1.61$  %ID/g at 30 min post-injection. Approximately 80% of the  $^{99\text{m}}\text{Tc}$ -CCMSH activity cleared out the body at 4 h post-injection (61). The favorable biodistribution of  $^{99\text{m}}\text{Tc}$ -CCMSH opened the avenue for using this class of metal-cyclized peptide radiopharmaceuticals for melanoma imaging and therapy.

Although  $^{99\text{m}}\text{Tc}$ -CCMSH exhibited high receptor-mediated melanoma uptake in B16/F1 melanoma-bearing mice, relatively high kidney uptake ( $14.60 \pm 1.88$  %ID/g at 4 h post-injection) was observed. It was critical to address this challenge to promote the clinical evaluation of this class of imaging probes for melanoma detection. It was postulated that non-specific renal activity accumulation was due to the electrostatic interaction between positively charged peptides and negatively charged surface of tubule cells, as the peptides were filtered in the glomerulus and reabsorbed in the cells of the proximal tubule (72). The strategies of infusing basic amino acids (lysine or arginine) and structural modification of the peptide (increasing the overall negative charge) were employed to decrease the renal uptake of radiolabeled peptides (62, 73, 74). The initial effort to reduce the non-specific kidney retention of the  $^{99\text{m}}\text{Tc}$ -CCMSH focused on co-injection of L-lysine with the  $^{99\text{m}}\text{Tc}$ -CCMSH and the substitution of Lys<sup>11</sup> with Gly or Nle (39). The co-injection of L-lysine decreased the renal uptake of  $^{99\text{m}}\text{Tc}$ -CCMSH by 50%. Compared to the co-injection of L-lysine, the substitution of Lys<sup>11</sup> with Gly or Nle in  $^{99\text{m}}\text{Tc}$ -CCMSH analogues exhibited more profound effects in reducing the renal uptakes of the peptides. However, the substitution of Lys<sup>11</sup> with Gly or Nle sacrificed the high tumor uptake values of the peptides (39), demonstrating that the Lys<sup>11</sup> residue in  $^{99\text{m}}\text{Tc}$ -CCMSH was critical to the melanoma targeting *in vivo*.

The success of the co-injection of L-lysine in renal uptake reduction demonstrated that the overall positive charge of the peptide plays an important role on the renal uptake of  $^{99\text{m}}\text{Tc}$ -CCMSH. Hence, more research on structural modification of Lys<sup>11</sup> was investigated to optimizing the peptide sequence of CCMSH (75). Five new CCMSH peptide analogues were synthesized by substituting Lys<sup>11</sup> with Arg, Orn, Met, Gln or Glu to examine the effects of positive charge distribution, structure of the side chain, neutral charge and negative charge at the 11<sup>th</sup> position on the tumor and renal uptakes of CCMSH peptide analogues (75). The introduction of a negative charged or neutral charged amino acid at the 11<sup>th</sup> position dramatically reduced the renal uptakes as well as tumor uptakes of the peptides. The positive charge at 11<sup>th</sup> position was responsible for the high tumor and renal uptakes of the peptides *in vivo*. Surprisingly, the substitution of Lys<sup>11</sup> with Arg<sup>11</sup> resulted in 30% higher tumor uptake and 43% less renal uptake of  $^{188}\text{Re}$ -(Arg<sup>11</sup>)CCMSH than that of  $^{188}\text{Re}$ -CCMSH (75). The increased tumor uptake and decreased renal uptake were likely attributed to the involvement of the amino acid at the 11<sup>th</sup> position in the receptor binding as well as the overall charge distribution of the peptide. Arg<sup>11</sup> appeared to be a

## Peptide radiopharmaceuticals for melanoma imaging



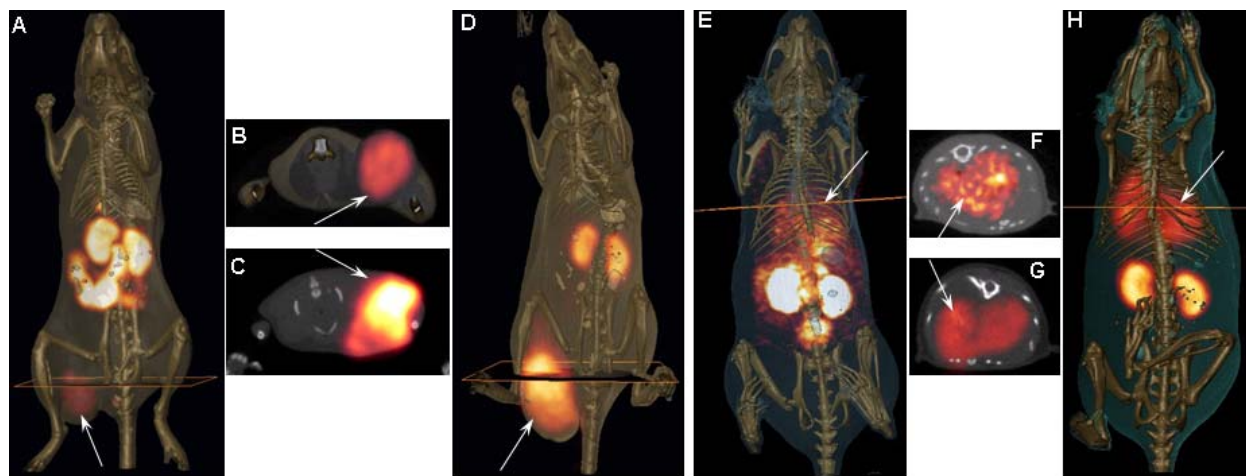
**Figure 2.** Schematic structures of metal-cyclized  $\alpha$ -MSH peptide analogues.

crucial amino acid which perfectly balanced the structure of the peptide.

Although [ $^{18}\text{F}$ ]FDG PET is the most commonly used radiopharmaceutical for the diagnosis and staging of melanoma, the detection of small melanoma metastases (<10 mm) remains challenging.  $^{99m}\text{Tc}(\text{Arg}^{11})\text{CCMSH}$  (Figure 2) was prepared to examine its melanoma targeting properties and pharmacokinetics in B16/F1 primary and B16/F10 metastatic melanoma-bearing mice (69).  $^{99m}\text{Tc}(\text{Arg}^{11})\text{CCMSH}$  exhibited high receptor-mediated tumor uptake ( $14.03 \pm 2.58$  %ID/g at 1 h post-injection) and fast

whole-body clearance ( $83.82 \pm 3.50$  %ID at 4 h post-injection) in B16/F1 primary melanoma-bearing mice. The  $^{99m}\text{Tc}(\text{Arg}^{11})\text{CCMSH}$  activity in metastatic melanoma-bearing lung was 5.32 times the activity in normal lung. Both primary and metastatic melanoma lesions were clearly visualized with  $^{99m}\text{Tc}(\text{Arg}^{11})\text{CCMSH}$  by Micro-SPECT/CT images, highlighting the potential of  $^{99m}\text{Tc}(\text{Arg}^{11})\text{CCMSH}$  as an effective imaging probe for primary and metastatic melanoma detection (Figure 3).

Radiohalogenated  $\alpha$ -MSH peptide analogues were re-investigated since metal cyclization had been



**Figure 3.** Whole body and transaxial images of  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  (A and B, respectively,) and  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  (D and C, respectively,) in B16/F1 flank melanoma-bearing C57 mice at 2 h post-injection. Whole body and transaxial images of  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  (E and F, respectively,) and  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  (H and G, respectively,) in B16/F10 pulmonary metastatic melanoma-bearing C57 mice 2 h post-injection. Reproduced by permission from the Society of Nuclear Medicine (69).

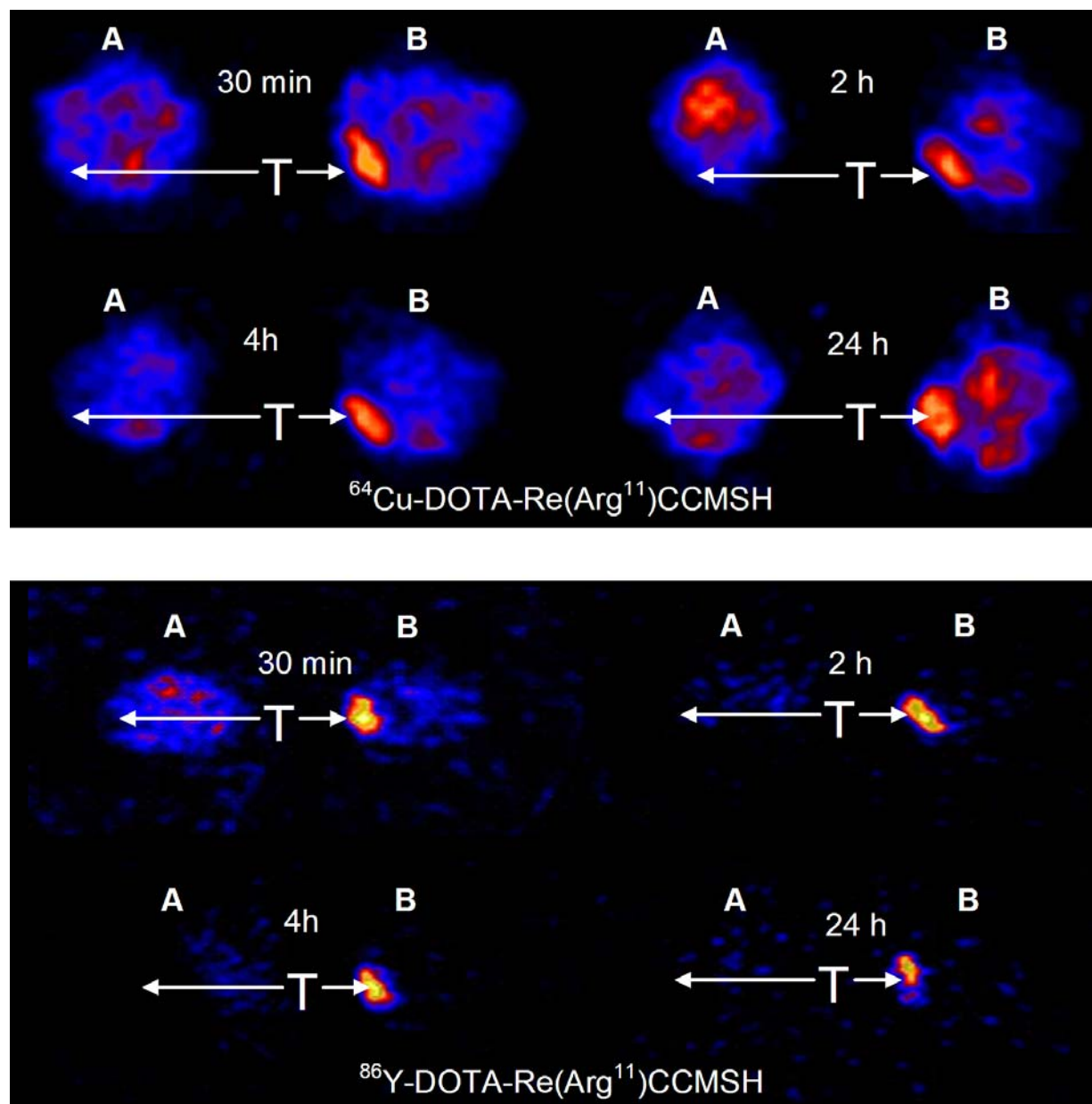
shown to enhance peptide stability and tumor retention. L-lysine and D-lysine were coupled to the N-terminus of the non-radioactive rhenium-cyclized CCMSH analogue  $[\text{Re}(\text{Arg}^{11})\text{CCMSH}]$  to yield  $\text{Ac-Lys-Re}(\text{Arg}^{11})\text{CCMSH}$  and  $\text{Ac-D-Lys-Re}(\text{Arg}^{11})\text{CCMSH}$  (71).  $\text{Ac-Lys-Re}(\text{Arg}^{11})\text{CCMSH}$  and  $\text{Ac-D-Lys-Re}(\text{Arg}^{11})\text{CCMSH}$  (Figure 2) were radiolabeled with  $^{125}\text{I}$  using the commercial reagent,  $^{125}\text{I-PIB}$ . Interestingly,  $\text{Ac-D-Lys}(\text{I}^{125}\text{-3- or 4-iodobenzoate (IBA))Re}(\text{Arg}^{11})\text{CCMSH}$  exhibited high melanoma uptake ( $17.69 \pm 4.13$  %ID/g at 2 h post-injection) and prolonged retention in tumor ( $7.18 \pm 2.14$  %ID/g at 24 h post-injection).  $\text{Ac-D-Lys}(\text{I}^{125}\text{-IBA})\text{-Re}(\text{Arg}^{11})\text{CCMSH}$  showed fast whole body clearance and low non-specific activity accumulation in normal organs (71). The enhanced tumor localization and prolonged retention of  $\text{Ac-D-Lys}(\text{I}^{125}\text{-IBA})\text{-Re}(\text{Arg}^{11})\text{CCMSH}$  was attributed to the rhenium cyclization and D-Lys incorporation. The favorable biodistribution of  $\text{Ac-D-Lys}(\text{I}^{125}\text{-IBA})\text{-Re}(\text{Arg}^{11})\text{CCMSH}$  highlighted the potential of  $^{18}\text{F}$ -labeled  $\text{Ac-D-Lys-Re}(\text{Arg}^{11})\text{CCMSH}$  as a melanoma-specific PET imaging probe for melanoma detection.

The metal chelator DOTA was coupled to the  $\text{Re}(\text{Arg}^{11})\text{CCMSH}$  to enable selective delivery of a wide variety of radionuclides for imaging and therapy (56, 63, 69), since DOTA can form stable complexes with a wide variety of diagnostic and therapeutic radionuclides. Non-radioactive rhenium was employed to cyclize the peptide.  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  (Figure 2) exhibited higher tumor uptake and prolonged retention than  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  (63, 69), however,  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  displayed faster renal clearance than  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  after 4 h post-injection. Pulmonary metastatic melanoma lesions were clearly imaged using micro-SPECT with both  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  and  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  at 2 h post-injection. Individual metastatic foci were not resolvable with  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$ , while several metastatic deposits were

identified with  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  (Figure 3). The higher imaging resolution of  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  is likely due to the superior imaging decay characteristics of  $^{99m}\text{Tc}$ . In combination with SPECT/CT equipment,  $^{99m}\text{Tc}-(\text{Arg}^{11})\text{CCMSH}$  and  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  could provide an effective and melanoma-specific approach to non-invasively monitor the development of tumors *in vivo*.

$\text{DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  was also successfully labeled with  $^{86}\text{Y}$  and  $^{64}\text{Cu}$  as PET imaging probes and evaluated for melanoma detection, since micro-PET allows high-resolution image and quantification of the radioactivity (64) (Figure 4).  $^{86}\text{Y}$ -labeled and  $^{64}\text{Cu}$ -labeled  $\text{DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  exhibited slightly lower melanoma uptakes than  $^{111}\text{In-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  due to possible differences in the vascularization of the melanoma tumors and the lower specific activity of  $^{64}\text{Cu}$ . However, the release of  $^{64}\text{Cu}$  from the  $^{64}\text{Cu-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  *in vivo* resulted in high liver uptake which might compromise its applications in the melanoma metastases detection in the liver (64). Hence, a cross-bridged cyclam chelator (CBTE2A) (Figure 2) was employed to substitute the DOTA to increase the stability of  $^{64}\text{Cu}$  coordination. The substitution of DOTA with CBTE2A dramatically reduced the  $^{64}\text{Cu}$  uptake in normal organs and greatly improved tumor to normal organ uptake ratios (70), demonstrating that CBTE2A chelator could form stable complex with  $^{64}\text{Cu}$  and solve the loss of  $^{64}\text{Cu}$  from DOTA *in vivo*. Small animal PET images of  $^{64}\text{Cu-CBTE2A-Re}(\text{Arg}^{11})\text{CCMSH}$  and  $^{86}\text{Y-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  showed that the primary melanoma lesions could be clearly visualized from 30 min to 24 h post-injection. Co-injection of 20  $\mu\text{g}$  of non-radioactive peptide blocked the tumor uptake of  $^{64}\text{Cu-CBTE2A-Re}(\text{Arg}^{11})\text{CCMSH}$  and  $^{86}\text{Y-DOTA-Re}(\text{Arg}^{11})\text{CCMSH}$  demonstrating MC1 receptor specificity (Figs. 4 and 5). These results demonstrated the potential of the metal-cyclized  $\alpha$ -MSH peptide analogues as PET imaging probes for early detection of malignant melanoma.





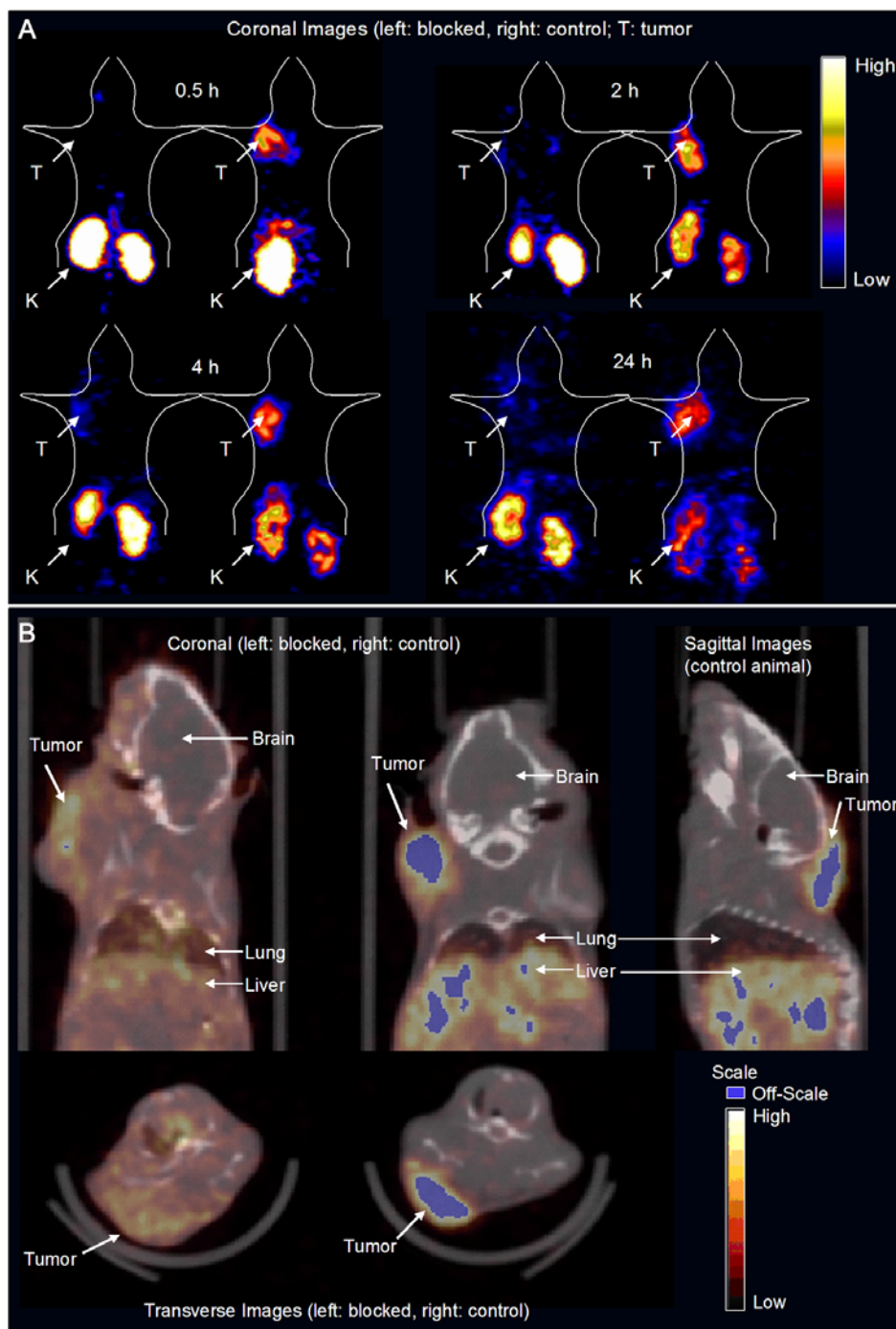
A = co-injected with 20 µg of DOTA-Re(Arg<sup>11</sup>)CCMSH; B = No blockade; T = tumor

**Figure 4.** Transaxial images of C57 mice implanted with B16F1 tumors 30 min, 2, 4 and 24 h after tail vein injection of 200 µCi of <sup>64</sup>Cu or <sup>86</sup>Y-DOTA-Re(Arg<sup>11</sup>)CCMSH. At each time point a mouse that received blockade (A, left) was co-imaged with a mouse that did not receive blockade (B, right). Blockade mice received 20 µg of DOTA-Re(Arg<sup>11</sup>)CCMSH. The PET images also showed that the administering of a blockade dose substantially reduced tumor uptake of both agents demonstrating that tumor uptake was receptor-mediated. It was also evident from the <sup>86</sup>Y- images that background accumulation was very low (i.e., low non-target tissue uptake) and that in the non-blockade mouse very little tracer accumulation was noted. Reproduced by permission from the Society of Nuclear Medicine (64).

## 5. CONCLUSIONS

Peptide-based radiopharmaceuticals, targeting receptors over-expressed on the surface of tumor cells, continue to receive great interest toward the development of cancer-specific diagnostic and therapeutic agents. The radiolabeled

α-MSH peptide analogues highlighted in this review are two types of representative examples targeting MC1 receptors over-expressed on melanoma cells. The results described in this review demonstrated the strategies used to develop radiolabeled α-MSH peptide analogues for melanoma targeting. Radiolabeled metal-cyclized α-MSH



**Figure 5.** (A) Coronal images of C57 mice implanted with B16F1 tumors at 30 min, 2, 4, and 24 h after tail vein injection of 150  $\mu$ Ci (500 ng) of  $^{64}\text{Cu}$ -CBTE2A-Re(Arg<sup>11</sup>)CCMSH. At each time point a mouse that received blockade (left) was co-imaged with a mouse that did not receive blockade (right). Blockade mice received 20  $\mu$ g of CBTE2A-Re(Arg<sup>11</sup>)CCMSH. The images shown were 1 mm slices, with the animal in the supine position, and the slices shown were through the center of the tumor volume. Since tumors did not grow in exactly the same location from animal to animal, the slices might show different tissues in addition to the tumors. The PET images showed that the administration of a blockade dose substantially reduced tumor uptake of the agent by such an extent that the tumor was difficult to delineate. It was also evident that the background accumulation was very low resulting in excellent tumor contrast. (T = tumor, K = kidney). (B) MicroPET/CT co-registered images of B16F1 tumor bearing mice 2 h after tail vein injection of 5.5 MBq (500 ng) of  $^{64}\text{Cu}$ -CBTE2A-Re(Arg<sup>11</sup>)CCMSH. The images shown were 1 mm slices and the slices shown were through the center of the tumor volume. Reproduced by permission from the Society of Nuclear Medicine (70).



peptide analogues exhibit great potential as melanoma-specific imaging probes for the clinic. Although the radionuclides involved in this review primarily focused on diagnostic radionuclides, it is important to note that these peptide analogues can be radiolabeled with therapeutic radionuclides for targeted radionuclide therapy. The results from radiolabeled  $\alpha$ -MSH peptide analogues and radiolabeled peptides for other receptors such as somatostatin, integrin, guanylin/guanylate cyclase-C and bombesin provide very strong evidence for the suitability and feasibility of their utilization in the early diagnosis and effective treatment of cancer. Early diagnosis and effective treatment will provide the cancer patients the best opportunities for cures or prolonged survival. Clearly, there is a great need of more effort and resources to develop peptide radiopharmaceuticals for cancer imaging and therapy.

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