Published in final edited form as:
*G Ital Dermatol Venereol*. 2010 April ; 145(2): 245–258.

# Targeted Melanoma Imaging and Therapy with Radiolabeled Alpha-Melanocyte Stimulating Hormone Peptide Analogues

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## Abstract

Radiolabeled alpha-melanocyte stimulating hormone (α-MSH) analogues have been used to define the expression, affinity and function of the melanocortin-1 receptor (MC1-R). The MC1-R is one of a family of five G-protein linker receptors, which is primarily involved in regulation of skin pigmentation. Over-expression of the MC1-R on melanoma tumor cells has made it an attractive target for the development of α-MSH peptide based imaging and therapeutic agents. Initially, the native α-MSH peptide was radiolabeled directly, but it suffered from low specific activity and poor stability. The addition of non-natural amino acids yielded α-MSH analogues with greater MC-1R affinity and stability. Furthermore, peptide cyclization via disulfide and lactam bond formation as well as site-specific metal coordination resulted in additional gains in receptor affinity and peptide stability in vitro and in vivo. Radiochemical stability of the α-MSH analogues was improved through the conjugation of metal chelators to the peptide's N-terminus or lysine residues for radionuclide coordination. In vitro cell binding studies demonstrated that the radiolabeled α-MSH analogues had low to subnanomolar affinities for the MC1-R. Biodistribution and imaging studies in the B16 mouse melanoma modeled showed rapid tumor uptake of the radiolabeled peptides, with the cyclic peptides demonstrating prolonged tumor retention. Cyclic α-MSH analogues labeled with beta and alpha emitting radionuclides demonstrated melanoma therapeutic efficacy in the B16 melanoma mouse model. Strong pre-clinical imaging and therapy data highlight the clinical potential use of radiolabeled α-MSH peptides for melanoma imaging and treatment of disseminated disease.

### Keywords

Melanoma; Peptide; Imaging; Therapy; α-MSH; Radionuclide

## Introduction

The development of melanoma specific radioimaging and radiotherapy agents would be beneficial for disease diagnosis, staging and timely treatment assessment. Currently, diagnostic radioimaging in melanoma management has been primarily limited to the use of non-melanoma specific imaging agents such as, $^{99m}$Tc-sulfur colloid, $^{99m}$Tc-human serum albumin and $^{99m}$Tc-MIBI for gamma scintigraphy and 2-[$^{18}$F]fluoro-2-deoxy-D-glucose ([$^{18}$F]FDG) for positron emission tomography (PET). Both $^{99m}$Tc-HSA and $^{99m}$Tc-sulfur

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colloid are routinely used to map the lymphatic drainage pattern of primary melanoma lesions as part of sentinel node detection and biopsy [1,2]. Non-invasive methods of imaging melanoma nodal metastases include the use of [$^{18}$F]FDG PET and $^{99m}$Tc-MIBI scintigraphy [3,4]. $^{18}$F-FDG has been examined extensively for its ability to diagnose and stage melanoma non-invasively [5–13]. Results from the imaging studies indicated that FDG PET has reasonable sensitivity and selectivity, with a clear positive correlation between tumor size and detection accuracy [14]. However, both $^{99m}$Tc-MIBI and $^{18}$FDG suffer from relatively high false positives rates of 15–17%. Moreover, it was reported that some melanoma cells were undetectable by [$^{18}$F]FDG since they used substrates other than glucose as energy sources [15].

In addition to the use of non-selective radiotracers in sentinel node detection and whole body scans, several melanoma specific probes have been examined for their tumor imaging properties in patients. Radiolabeled monoclonal antibodies such as NR-ML-05 [16] and 9.2.2 [17], have been radiolabeled with $^{99m}$Tc for imaging melanoma. Unfortunately, results in the clinic have been disappointing and have not resulted in the routine clinical use of radiolabeled antibodies for melanoma detection. Several small molecule benzamide-based imaging agents that target melanin have undergone limited trials in patients or are in the planning stages [17–20]. Most of the benzamide derivatives are radioiodinated and exhibit some in vivo deiodination and liver metabolism. While this class of compound has demonstrated promising imaging potential in melanoma tumors, many benzamide compounds exhibit liver metabolism and limited uptake in amelanotic tumors. The results from ongoing clinical trails will ultimately determine the utility of this class of compound for melanoma imaging. Peptides that target melanoma specific antigens are also being examined as radioimaging and radiotherapeutic agents. One of the most widely targeted melanoma antigens is the melanocortin-1 receptor (MC1-R), overexpressed on melanoma cells [21,22]. Radiolabeled alpha-melanocyte stimulating hormone (α-MSH) peptide analogues, which target the MC1-R for melanoma imaging and therapy, are the subject of this review.

The native α-MSH peptide hormone (Ac-Ser$^1$-Tyr$^2$-Ser$^3$-Met$^4$-Glu$^5$-His$^6$-Phe$^7$-Arg$^8$-Trp$^9$-Gly$^{10}$-Lys$^{11}$-Pro$^{12}$-Val$^{13}$-$NH_2$), proteolytically processed from proopiomelanocortin, is primarily responsible for regulation of skin pigmentation [23,24]. Alpha-MSH peptides bind the melanocortin-1 receptor (MC1-R) selectively with nanomolar to subnanomolar affinities [25,26]. Numerous α–MSH analogues have been developed with high affinities and specificities for α–MSH receptors [27] (Table 1). Many potent α–MSH analogues contain non-natural amino acid insertions that enhance receptor binding by stabilizing the bioactive conformation of the peptide [28]. The most widely used α-MSH analogue is the linear Ac-Ser$^1$-Tyr$^2$-Ser$^3$-Nle$^4$-Glu$^5$-His$^6$-D-Phe$^7$-Arg$^8$-Trp$^9$-Cys$^{10}$-Lys$^{11}$-Pro$^{12}$-Val$^{13}$-$NH_2$ (Ac-[Nle$^4$, D-Phe$^7$]-α-MSH, NDP) analogue, which is referred to as the gold standard due to its superior (picomolar) receptor affinity and protease resistance properties [29]. In addition, α–MSH analogues employing disulfide bond [30], lactam bond [31] and site-specific rhenium coordination [32] have yielded conformationally constrained peptide structures that exhibit enhanced biological activity and receptor affinity. Truncated NDP analogues, containing the minimal His-(d)Phe-Arg-Trp pharmacophore, have also been examined as melanoma targeting vehicles [33,34].

Alpha-MSH peptides bind the MC1-R, which is one of five receptor isoforms that make up the melanocortin receptor family [35,36]. The MC1-R is a seven-helix transmembrane G-protein linked receptor, which mediates its physiological responses via cyclic adenosine monophosphate (cAMP) dependent signaling pathways [24]. Upon agonist binding, the MC1-R peptide-receptor complex is rapidly internalized [25]. Radio-ligand binding studies demonstrated the presence of MC1-Rs on the surfaces of human malignant melanomas,

making them attractive molecular targets for melanoma targeting [21]. Functional receptor populations range from 1,000–6,000 on human melanotic and amelanotic melanoma cells and 5,000–8,000 receptors on murine melanoma cells [21,22]. The high affinities of α-MSH and its analogues for the MC1-R make them attractive vehicles for melanoma-targeted radioimaging and radiotherapy, however, the relatively low number of receptors per cell demands that the radiopharmaceuticals have high specific activities.

This review will highlight the development of radiolabeled MC1-R targeting α-MSH peptide analogues for melanoma imaging and treatment. The chemical structures, molecular weights and in vitro cell binding $IC_{50}$ values for the MC1-R targeting peptides are presented in Table 1. First, a brief historical look at the development of radiolabeled α-MSH peptide analogues will be presented. Second, the development melanoma imaging agents will be presented. The radiolabeled peptides will be broken into single photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging agents. A presentation of MC1-R targeted radiotherapeutic peptides will follow. The radiolabeled peptides developed for melanoma treatment will be subdivided into beta and alpha emitters. A comparison of the imaging and therapeutic properties of the radiolabeled peptides will be limited to the B16 melanoma model since this animal model is common amongst the peptides reviewed. Finally, the prospects and challenges facing the clinical translation of the radiolabeled melanoma imaging and therapeutic agents will be discussed.

Historically, initial research efforts were focused on the development of tritium-labeled linear α-MSH analogues [37–39], such as Ac-[($^3H_4$)Nva$^{1,4,13}$, D-Phe$^7$]-α-MSH, was reported to be suitable for in vivo tissue distribution and stability studies. However, the tritium-labeled analogues suffered from relatively low specific activity (12.21 GBq/μmol), making in vivo tumor targeting inefficient [40,41]. The specific activities of MC1-R targeting peptides were dramatically increased by labeling them with $^{125}$I on Tyr$^2$ (41). The preparation and characterization of Ac-[$^{125}$I-Tyr$^2$]-α-MSH, Ac-[$^{125}$I-Tyr$^2$, Nle$^4$]-α-MSH and Ac-[$^{125}$I-Tyr$^2$, Nle$^4$, D-Phe$^7$]-α-MSH in receptor binding assays were reported in the literature [21,42–45]. However, the use of the $^{125}$I-labeled α-MSH analogues was mainly limited to in vitro studies due to dehalogenation. Several new approaches were developed to reduce *in vivo* dehalogenation. It was reported that Ac-[Nle$^4$, D-Phe$^7$]-α-MSH radiolabeled with *N*-succinimidyl 3-iodobenzoate (SIB) or *N*-succinimidyl 4-iodobenzoate (PIB) at Lys$^{11}$ was inert to the *in vivo* dehalogenation [46]. Ac-[Nle$^4$, D-Phe$^7$, $^{125}$I-IBA-Lys$^{11}$]-α-MSH exhibited higher receptor binding affinity than Ac[$^{131}$I-Tyr$^2$, Nle$^4$, D-Phe$^7$]-α-MSH [48] and reduced radioactivity in the thyroid and stomach of mice, demonstrating its resistance to *in vivo* dehalogenation. Likewise, *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate (FBA) labeled Ac-[Nle$^4$, D-Phe$^7$]-α-MSH exhibited rapid clearance with little evidence for defluorination in normal mice [47]. No biodistribution of Ac-[Nle$^4$, D-Phe$^7$, $^{125}$I-IBA-Lys$^{11}$]-α-MSH and Ac-[Nle$^4$, D-Phe$^7$, $^{18}$F-FBA-Lys$^{11}$]-α-MSH was reported in melanoma-bearing mice.

## I. MC1-R avid melanoma imaging agents

### A. SPECT imaging agents

Many linear radiolabeled Ac-[Nle$^4$, D-Phe$^7$]-α-MSH (NDP) peptide analogues have been developed over the years as potential melanoma imaging agents (Table 1). Typically a radionuclide chelator such as diethylenetriamene pentaacetate (DTPA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), mercato-acetylglycylglycine ($MAG_2$) or chelator amino acid sequence like Cys-Gly-Cys-Gly would be appended to the targeting peptide sequence at its amino terminus or at a lysine residue [48,49]. $MAG_2$ and Cys-Gly-Cys-Gly are $N_3S$ and $N_2S_2$ chelation systems that coordinate $^{99m}$Tc, while DTPA and DOTA and well suited for $^{111}$In coordination [50,51]. Technetium-99m is an ideal SPECT imaging radionuclide with an imageable 140 keV gamma emission and a 6 h half-

life, while $^{111}$In is nearly as good with a 171 keV gamma emission and a 2.8 day half-life. Moderate energy gamma emissions coupled with half-lives that closely match the in vivo half-life of the targeting molecules make $^{99m}$Tc and $^{111}$In the most popular SPECT imaging radionuclides. $^{99m}$Tc-CGCG-NDP exhibited greater tumor to blood and tumor to muscle ratios than $^{99m}$Tc-MAG$_2$-NDP and $^{125}$I-Tyr$_2$-NDP-MSH at 30 min, 1 and 4 h post-injection [51]. However, the moderate melanoma uptake of $^{99m}$Tc-CGCG-NDP (6.52±1.11 %ID/g at 30 min post-injection) prevented its further evaluation. A novel chelating derivative of α-MSH was synthesized in which two α-MSH sequences were joined at their N-termni by the DTPA chelator to yield (bis)MSH-DTPA. Indium-111 labeled (bis)MSH-DTPA exhibited approximately 3 % ID/g in melanoma mice 24 h postinjection, with low levels of uptake in normal tissues except for the kidney [52]. In a limited clinical study, $^{111}$In labeled (bis)MSH-DTPA was reported to image melanoma with 89% sensitivity [53]. This report represents one of the few clinical studies to be published using a radiolabeled MC1-R targeting peptide.

A head to head comparison of the biodistribution and tumor targeting properties of several DOTA conjugated NDP peptide analogues was investigated. $^{111}$In-DOTA-MSH$_{OCT}$, a truncated version of the NDP peptide with DOTA conjugated to its N-terminus, exhibited 4.31±0.30 % ID/g and 1.17±0.13 % ID/g at 4 and 24 h post-injection in B16/F1 melanoma-bearing mice [54]. Although $^{111}$In-DOTA-MSH$_{OCT}$ exhibited significant ($p<0.05$) lower tumor uptake than $^{111}$In-DOTA-NDP-MSH at 4, 24 and 48 h postinjection, the accumulation of $^{111}$In-DOTA-MSH$_{OCT}$ radioactivity in normal organs was lower than that of $^{111}$In-DOTA-NDP-MSH at all time points except for the kidneys [54]. $^{111}$In-DOTA-NAPamide displayed tumor uptake of 7.56±0.51 % ID/g and the kidney uptake of 5.06±0.32 % ID/g at 4 h post-injection [34]. The DOTA was coupled to the side chain of Lys on the C-terminus of the NAPamide peptide and its N-terminus was acetylated. These changes resulted in the improvement of the tumor to kidney uptake ratio of the $^{111}$In-DOTA-NAPamide compared to the $^{111}$In-DOTA-MSH$_{OCT}$. The substitution of $^{111}$In with $^{67}$Ga further improved the tumor to kidney ratio of $^{67}$Ga-DOTA-NAPamide, highlighting the potential of $^{68}$Ga-DOTA-NAPamide for melanoma PET imaging [34].

Recently, the advent of chelators and kit preparations for $^{99m}$Tc(CO)$_3$ fostered the facile and stable labeling of α-MSH analogues [55]. A truncated NDP analogue conjugated with pyrazolyl-diamine backbone (pz) on the C-terminal lysine, Ac-[Nle$^4$, Asp$^5$, D-Phe$^7$, Lys$^{11}$(pz)]-α-MSH, was labeled with $^{99m}$Tc(CO)$_3$ and investigated for its tumor imaging properties. Ac-[Nle$^4$, Asp$^5$, D-Phe$^7$, Lys$^{11}$(pz-$^{99m}$Tc(CO)$_3$)]-α-MSH exhibited good tumor uptake in the B16/F1 melanoma model with 4.24±0.94 % ID/g in the tumor at 4 h postinjection. Non-specific uptake in the kidneys was moderate at 4.50±2.40 % ID/g, however, uptake was 6.7±2.1% ID/g in the liver and 14.4±1.6 % ID/g in the intestines. While SPECT imaging reveal good tumor localization, the biodistribution of the $^{99m}$Tc(CO)$_3$ labeled peptide appeared to be dominated by the hydrophobic $^{99m}$Tc-tricarbonyl moiety as evidenced by the hepatobiliary-gastrointestinal route of excretion.

Although linear radiolabeled Ac-[Nle$^4$, D-Phe$^7$]-α-MSH (NDP) peptide analogues were engineered for increased stability in vivo, they have suffered from moderate tumor uptake and poor tumor retention. Tumor uptake could be adversely affected by conjugation of the radionuclide chelator or prosthetic group, while retention was related to the stability of the radionuclide coordination and peptide turnover once internalized. To improve in vivo stability and tumor to normal tissue uptake structurally constrained α-MSH peptide analogues were designed that were cyclized by metal coordination [32] or by disulfide [30] or lactam [31] bond formation (Table 1).

A novel α-MSH peptide analogue (CCMSH), that was cyclized by $^{99m}$Tc, $^{188}$Re, or Re, coordination, was developed for melanoma imaging and therapy [32,56]. The metal cyclized portion of the peptide contained the optimal consensus MC1-R binding sequence (His-D-Phe-Arg-Trp) of the superpotent α-MSH analogue NDP yielding similar cell and MC1-R binding properties [32,56]. $^{99m}$Tc-CCMSH exhibited rapid high tumor uptake and fast whole-body clearance in B16/F1 melanoma-bearing mice. The tumor uptake was 10.88±0.54 % ID/g and 1.38±0.36 % ID/g at 0.5 and 24 h post-injection. Although $^{99m}$Tc-CCMSH exhibited high receptor mediated melanoma, it demonstrated relatively high kidney uptake of 14.60±1.88 % ID/g at 4 h post-injection. A DOTA conjugated analogue of metal cyclized CCMSH, DOTA-ReCCMSH was developed to broaden the number of radioisotopes that could be targeted to melanoma tumors [57]. Rhenium cyclization was maintained since it was demonstrated that metal cyclization enhanced peptide stability as well as tumor uptake and retention [32]. $^{111}$In-DOTA-ReCCMSH exhibited similar initial tumor uptake but demonstrated longer retention 4.86±1.52 % ID/g versus 1.38±0.36 % ID/g of $^{99m}$Tc-CCMSH 24 h postinjection [57]. Non-specific renal retention of $^{111}$In-DOTA-ReCCMSH at 4 h postinjection was 9.27±2.65 % ID/g, which was lower than 14.60±1.88 % ID/g for $^{99m}$Tc-CCMSH. To address the high kidney uptake issue a series of amino acid substitutions, outside the core receptor binding sequence, were examined. Substitution of Lys$^{11}$ to Arg$^{11}$ yielded the greatest reduction in non-specific kidney uptake and improved tumor uptake [58]. The resulting peptide, $^{111}$In-DOTA-Re(Arg$^{11}$)CCMSH, displayed the very high tumor uptake of 17.41±5.61 % ID/g and 5.64±0.52 % ID/g at 4 and 24 h postinjection. Kidney uptake at 4 h postinjection was 7.37±1.31 % ID/g. SPECT imaging studies with $^{99m}$Tc-(Arg$^{11}$)CCMSH and $^{111}$In-DOTA-Re(Arg$^{11}$)CCMSH yielded excellent primary and metastatic tumor images in the B16/F1 and B16/F10 mouse melanoma models, respectively [59].

Alpha-MSH peptides, cyclized by lactam bond formation, also demonstrated improve in vivo stability and high melanoma tumor uptake. A Lys$^{3}$-Asp$^{14}$ lactam bridge was used to cyclize the peptide (CycMSH), while the radiometal chelator DOTA was coupled to the N-terminus of the cyclic peptide with or without an amino acid (-Gly-Glu-) linker [60]. The unique lactam bridge-cyclization made the radiolabeled α-MSH peptides stable both *in vitro* and *in vivo*. Compared to $^{111}$In-DOTA-CycMSH, the introduction of the –Gly-Glu- linker decreased the renal uptake of $^{111}$In-DOTA-GlyGlu-CycMSH by 44% without affecting the melanoma uptake at 4 h post-injection [60]. $^{111}$In-DOTA-GlyGlu-CycMSH displayed high receptor-mediated melanoma uptake (10.40±1.40 % ID/g at 2 h post-injection) in B16/F1 melanoma-bearing C57 mice. Both B16/F1 flank melanoma and B16/F10 pulmonary metastatic melanoma could be clearly visualized by small animal SPECT/CT using $^{111}$In-DOTA-GlyGlu-CycMSH as an imaging probe [60,61], highlighting the potential applications of radiolabeled lactam bridge-cyclized α-MSH peptides as effective melanoma-specific diagnostic agents for melanoma. A lactam cyclized α-MSH analogue, based on the sequence of melanotan II [62], was synthesized for labeling with $^{99m}$Tc(CO)$_3$. A pyrazolyl-diamine backbone chelator was appended to the N-terminus of the peptide for $^{99m}$Tc(CO)$_3$ chelation. The cyclic radioconjugate displayed high tumor uptake of 9.26±0.83 % ID/g and 11.31±1.83 % ID/g at 1 and 4 h postinjection injection in the B16/F1 melanoma mouse model. The linear version of the cyclic radioconjugate exhibited much less tumor uptake with 0.99±0.08 % ID/g 4 h postinjection underscoring the importance of peptide cyclization on tumor uptake and retention.

Many promising α-MSH peptide agonists that bound the MC1-R for melanoma imaging have been developed and tested at the preclinical stage. The inclusion of non-natural amino acids into the targeting peptide sequences dramatically improved their in vivo stability. Short linear α-MSH peptides tend to have very rapid in vivo pharmacokinetics, punctuated by moderate tumor uptake very low normal tissue uptake and fast clearance yielding good

tumor to normal tissue ratios. Cyclic α-MSH peptides displayed higher tumor uptake and retention than their linear counterparts. Moreover, clearance of the cyclic radiolabeled peptides from normal tissues was rapid as whole body disappearance of radioactivity. Another important factor for obtaining high tumor to normal tissue ratios and optimal melanoma images is high specific activity radiolabeled peptide preparation. High performance liquid chromatography purification of the final radiolabeled product was necessary to obtain high specific activity imaging agent preparation. Low specific activity preparations will result in low to no tumor uptake no matter what targeting peptide is used.

## B. PET imaging agents

PET imaging offers distinct advantages over other forms of functional imaging with respect to sensitivity, resolution and quantitation [63]. Coupling the outstanding imaging properties of PET with selective tumor-targeting properties of peptide based receptor ligands, offers an exciting opportunity for highly sensitive tumor specific imaging. The development of α-MSH analogues labeled with positron (β+) emitting radionuclides has mirrored SPECT agent development. Initially, the NDP peptide was labeled with $^{18}$F-para-fluorobenzoate (PFB) on Lys$^{11}$ to yield Ac-[Nle$^{4}$, D-Phe$^{7}$, Lys$^{11}$($^{18}$F-PFB)]-α-MSH. The peptide exhibited very low normal tissue uptake and very rapid whole body clearance [47]. Unfortunately, no biodistribution data was present in B16/F1 tumor bearing mice. A truncated form of the linear NDP peptide, NAPamide, was labeled with the positron emitting radionuclides $^{64}$Cu ($t_{1/2}$ = 12.7 h, 17.4% β+, 41% EC, 40% β−, $E_{avg}$ = 278 keV), $^{68}$Ga ($t_{1/2}$= 68 min, β+ = 88%, $E_{avg}$ = 1.89 MeV) and $^{18}$F ($t_{1/2}$ = 109.7 min, 97% β+, $E_{avg}$ = 250 keV). $^{64}$Cu and $^{67}$Ga (surrogate for $^{68}$Ga) labeled DOTA-NAPamide exhibited tumor uptake values of 4.43 ± 0.94 % ID/g [64] and 9.43±1.06 % ID/g [34] 4 h postinjection in the B16/F10 and B16/F1 melanoma mouse models, respectively. The observed high liver uptake of $^{64}$Cu-DOTA-NAPamide was likely due to the loss of $^{64}$Cu from the DOTA chelator in vivo and contributed to a lower tumor uptake [64]. Both $^{64}$Cu and $^{68}$Ga labeled DOTA-NAPamide yield clear melanoma PET imaging as soon as 1 h postinjection. Interestingly, $^{18}$F-fluorobenzoate (FB) labeled NAPamide showed 1.19±0.11 % ID/g and 0.25±0.05 % ID/g uptake in the B16/F10 melanoma model at 1 h and 4 h postinjection [65]. Despite the low tumor uptake melanoma PET images were acquired 1 h postinjection.

The ability of the rhenium cyclized DOTA-Re(Arg$^{11}$)CCMSH peptide to target a number of metallic positron emitting radionuclides to melanoma tumors was investigated in the B16/F1 mouse model. DOTA-Re(Arg$^{11}$)CCMSH was radiolabeled with divalent $^{64}$Cu, $^{86}$Y ($t_{1/2}$=14.7 h, β+ = 33%, $E_{avg}$ = 278 keV) [66] and $^{68}$Ga [67]. Solid tumor uptake of $^{64}$Cu-DOTA-Re(Arg$^{11}$)CCMSH was 7.35±1.47 % ID/g at 4 h, however, liver uptake was nearly identical at 7.34±1.79 % ID/g. Imaging studies revealed excellent tumor visualization but also reflected the liver accumulation of $^{64}$Cu. The tumor images were very encouraging but the loss of $^{64}$Cu from DOTA was concerning. The PET imaging properties of $^{86}$Y-DOTA-Re(Arg$^{11}$)CCMSH [66] and $^{68}$Ga-DOTA-Re(Arg$^{11}$)CCMSH [67] were also examined in the B16/F1 mouse melanoma model. Yttrium-86 was cyclotron produced while $^{68}$Ga was obtained from a Ge-68/Ga-68 generator. Tumor uptake of $^{86}$Y-DOTA-Re(Arg$^{11}$)CCMSH was 9.83±2.27 % ID/g and 9.98±2.05 % ID/g at 2 and 4 h postinjection in the B16/F1 model, while the tumor uptake for $^{68}$Ga-DOTA-Re(Arg$^{11}$)CCMSH was 4.31±1.94 % ID/g and 4.24±1.41 % ID/g 0.5 h and 2 h postinjection. Differences in tumor uptake at 2 h postinjection were attributed to the differences in specific activities of the peptide preparations. The specific activity for $^{86}$Y-DOTA-Re(Arg$^{11}$)CCMSH was ~3000 mCi/mg, while the specific activity for $^{68}$Ga-DOTA-Re(Arg$^{11}$)CCMSH was only 50 mCi/mg. Contaminating metals eluted from the Ge-68/Ga-68 generator were implicated in the loss of radiolabeling efficiency and reduction in specific activity. Improved tumor uptake of $^{68}$Ga-

DOTA-Re(Arg$^{11}$)CCMSH, 6.27±1.60 % ID/g at 1 h postinjection, was observed with higher specific activity $^{68}$Ga in which contaminating metals were removed [68].

A new chelator, CBTE2A, which had superior copper chelating abilities was conjugated to Re(Arg$^{11}$)CCMSH in an attempt to improve the radiochemical stability of the $^{64}$Cu-labeled peptide [69]. The tumor uptake of $^{64}$Cu-CBTE2A-Re(Arg$^{11}$)CCMSH was identical to its DOTA homolog but the amount of radioactivity in the liver was significantly less (1.74±0.52 % ID/g VS 7.34±1.79 % ID/g) at 4 h. The greatly reduced liver activity was apparent in the imaging study (). In vitro cell binding experiments demonstrated that both DOTA and CBTE2A Re(Arg$^{11}$)CCMSH peptides had low nanomolar affinities for the MC1-R and were rapidly internalized. However, $^{64}$Cu-CBTE2A-Re(Arg$^{11}$)CCMSH exhibited superior in vivo radiochemical stability and tumor to normal tissue uptake values. Tumor to blood, muscle and skin ratios of $^{64}$Cu-CBTE2A-Re(Arg$^{11}$)CCMSH were superior to $^{68}$Ga-DOTA-Re(Arg$^{11}$)CCMSH and $^{64}$Cu-DOTA-Re(Arg$^{11}$)CCMSH, and equivalent to $^{86}$Y-DOTA-Re(Arg$^{11}$)CCMSH. In addition to peptide stability and receptor affinity, the specific activity of the radiolabeled peptide preparation was shown to be critical for high tumor uptake. For example, B16/F1 tumor uptake of $^{64}$Cu-CBTE2A-Re(Arg$^{11}$)CCMSH was 7.09±3.20 % ID/g at 600mCi/mmol and 22.59±2.99 % ID/g at 5000 mCi/mmol 2 h post injection emphasizing the importance of high specific activity for optimal imaging.

Recently, Ac-dLys$^{2}$-Re(Arg$^{11}$)CCMSH was radiolabeled with $^{18}$F-FB on Lys$^{2}$ and examined for its tumor targeting and imaging properties in the B16/F1 and F10 melanoma mouse models. Tumor uptake of Ac-dLys$^{2}$($^{18}$F-FB)-Re(Arg$^{11}$)CCMSH was 2.11±0.12 % ID/g and 0.83±0.05 % ID/g at 2 and 4 h postinjection in the B16/F10 model [70] and 0.43±0.08 % ID/g and 0.27±0.07 % ID/g at the same time points in the B16/F1 model (unpublished data). Similarly low tumor uptake data was observed between $^{18}$F-FB-NAPamide [65] and Ac-dLys$^{2}$($^{18}$F-FB)-Re(Arg$^{11}$)CCMSH [70]. In vitro $IC_{50}$ studies with the $^{19}$F (stable isotope) labeled peptides demonstrated that they retained their receptor targeting properties and displayed high affinities for cultured melanoma cells. Interestingly, Ac-dLys$^{2}$($^{125}$I-IBA)-Re(Arg$^{11}$)CCMSH demonstrated very high tumor uptake values of 17.69±4.13 % ID/g and 15.10±1.38 % ID/g [71]. Either something in the $^{18}$F labeling process is destroying the activity of MC1-R targeting peptides or $^{18}$F-fluorination alters the in vivo biodistribution properties so dramatically as to prevent tumor access. Clearly new $^{18}$F-peptide labeling strategies are needed to take advantage of the radionuclide with the best PET imaging properties.

## II. MC1-R avid melanoma radiotherapy agents

Receptor-avid peptides can be deployed as effective delivery vehicles to selectively and specifically target cytotoxic radiation generated from radionuclide decay to tumor cells [72]. Theoretically, peptide-targeted radionuclide therapy can specifically deliver the radiation dose to tumor cells, while sparing the normal tissues and organs. Beta-particle-emitting, alpha-particle-emitting and Auger-electron-emitting radioisotopes may be used to radiolabel receptor-targeting peptides for targeted radionuclide therapy [73]. High-energy beta-emitters such as $^{188}$Re and $^{90}$Y appear appropriate for the treatment of larger tumors or large tumor burdens. Medium- and lower- energy beta emitters, such as $^{177}$Lu, may be more suitable for treating smaller tumors and metastatic deposits [74–77]. Alpha emitters ($^{225}$Ac, $^{212}$Pb $^{213/212}$Bi, $^{211}$At) are attractive for treating small tumor and metastases due to their short path-length and high linear energy transfer (LET) [78]. Auger-emitters ($^{111}$In) appear to have favorable properties for treating metastases and disseminated tumor cells due to their highly localized energy deposition and very short path-lengths [79].

Selection of the therapeutic radionuclide is dependent on its physical decay properties and the biological half-life of the targeting peptide [80]. The radionuclides examined for peptide-targeted melanoma therapy have relatively short half-lives from several hours to several days. For optimal targeted radiation dose deposition, the half-life of the radionuclide should be similar to the *in vivo* biological half-life of the peptide in the tumor. Radionuclides with very short half-lives present formulation and administration hurdles that may limit their abilities to target an optimal radiation dose to the tumor, while radionuclides with long half-lives may not achieve a high enough dose rate over a long enough period of time to insure tumor cell death. The use of targeted radionuclide "nanogenerators" was employed to significantly improve the targeted dose of the short half-life alpha-emitters $^{213}$Bi [81] and $^{212}$Bi [82]. By targeting parent radionuclides with longer half-life, high tumor doses of the alpha-emitting radionuclides were achieved, resulting in high therapeutic efficacies. Long-lived radionuclides (i.e. $^{177}$Lu) would benefit from the strategies of improving tumor retention such as cellular internalization of the radiolabeled peptide or enhancing tumor-binding affinities by using multi-valent peptides for increase avidity. The energies and ranges of particles emitted by therapeutic radionuclides are also important considerations in peptide-targeted radionuclide therapy. High-energy beta particle-emitters like $^{90}$Y and $^{188}$Re have long maximum path-lengths, which permit the irradiation of several layers of tumor cells [58]. Particles that traverse multiple layers of cells yield cross-fire effects, in which tumor cells are irradiated from radionuclides bound to adjacent or nearby cells. Cross-fire effects establish a homogeneous radiation field in the tumor that can overcome targeting antigen expression heterogeneity. The high energies and longer particle path-lengths of $^{90}$Y and $^{188}$Re appear to be best suited for larger tumors or large tumor burdens, enabling deposition of the particle's ionizing energy within the confines of the tumor volume [83–84]. Short pathlength radionuclides such as alpha emitters can traverse 3–5 cells making them attractive for treating small metastatic deposits. Alpha emitter deposit large amounts of energy over pathlenght of 60–80 micrometers resulting in cell death from 2–3 alpha-particle traversals [85]. To be optimally effective very short pathlength Auger emitters like $^{111}$In would have to be targeted to the nuclei of tumor cells.

The first radiolabeled peptide therapy study targeting the MC1-R was performed with $^{188}$Re-(Arg$^{11}$)CCMSH [86]. The radionuclide $^{188}$Re was selected for its high-energy beta-particle emission, coordination chemistry, imageable gamma-emission and because its half-life ($t_{1/2}$=17 h) was consistent with the biological half-life of the peptide targeting vector in the tumor. High specific activity $^{188}$Re was obtained from a $^{188}$W/$^{188}$Re radionuclide generator (Oakridge National Laboratory, Oakridge, TN). Site-specific coordination of $^{188}$Re via the Cys$^{3,4,10}$ thiols and Cys$^{4}$ amide nitrogen resulted in the formation of the radiolabeled metal-cyclized peptide [32]. Tumor uptake values at 1 h, 4 h and 24 h were 20.44±1.91 % ID/g, 16.37±3.27 % ID/g and 3.50±2.32 % ID/g, respectively. Accumulation and retention of the peptide-targeted $^{188}$Re was clearly visualized in flank tumors, highlighting the feasibility of utilizing gamma or SPECT imaging to monitor $^{188}$Re-(Arg$^{11}$)CCMSH biodistribution and to calculate individual patient-specific dosimetry *in vivo*. Therapy studies were performed in C57 mice bearing B16/F1 syngeneic murine melanoma tumors [87]. Palpable dark-colored melanoma tumors were identifiable 3 days post-tumor cell implantation. Groups of 10 tumor-bearing mice were treated with a saline placebo, 7.4 MBq, 22.2 MBq or 2 × 14.8 MBq of $^{188}$Re-(Arg$^{11}$)CCMSH on day 4 and 7 (2nd dose of 2 × 14.8 MBq). In contrast to the saline placebo control group, all $^{188}$Re-(Arg$^{11}$)CCMSH treatment groups showed substantial inhibition of tumor growth during the period of therapy studies. A single dose administration of 22.2 MBq of $^{188}$Re-(Arg$^{11}$)CCMSH resulted in a more pronounced delay in tumor growth than a single 7.4MBq dose. Mice receiving 2 × 14.8MBq doses of $^{188}$Re-(Arg$^{11}$)CCMSH exhibited the best overall tumor growth inhibition. The mean survival times for the groups of mice treated with 7.4 MBq, 22.2MBq and 2 × 14.8MBq of $^{188}$Re-(Arg$^{11}$)CCMSH were 10.2±1.0 days, 10.3±1.3 days and 13.3±1.9 days, respectively,

compared to a mean survival time of 9.4±1.1 days for the control group. Although single dose administration of $^{188}$Re-(Arg$^{11}$)CCMSH resulted in tumor growth rate reduction, it did not significantly extend the mean survival times of the treatment group over the control mice. Only the multi-dose regimen resulted in a significant ($P < 0.05$) improvement in mean survival time over the control group. No evidence of radiation-related damage to the kidneys was observed in the treatment groups upon postmortem histopathology analysis. It was likely that the large β-particle range of $^{188}$Re resulted in the deposition of radiation outside the primary tumor volume resulting in moderate but significant treatment efficacy in the B16/F1 solid tumor model.

The biodistribution and therapeutic efficacy of $^{177}$Lu-DOTA-Re(Arg$^{11}$)CCMSH were examined in B16/F1 tumor-bearing mice [88,89]. DOTA-Re(Arg$^{11}$)CCMSH was developed to allow one to target a large number of diagnostic and therapeutic radionuclides to melanoma tumor using the exact same targeting vehicle. The medium energy β-emitter $^{177}$Lu was obtained from the University of Missouri Research Reactor (MURR, Columbia MO) for DOTA-Re(Arg$^{11}$)CCMSH labeling. Biodistribution studies of $^{177}$Lu-DOTA-Re(Arg$^{11}$)CCMSH showed high and prolonged tumor uptake and rapid whole-body clearance of radioactivity [88]. Tumor uptake values at 2 h, 4 h and 24 h were 14.48±0.85 % ID/g, 17.68±3.32 % ID/g and 9.05±4.31 % ID/g, respectively. Radioactivity clearance from the normal organs and tissues was rapid with the exception of the kidney. Greater than 90% of the injected radioactivity was cleared into the urine by 2 h. The kidney uptakes of $^{177}$Lu-DOTA-Re(Arg$^{11}$)CCMSH were 17.99±2.47 % ID/g, 19.09±2.38 % ID/g and 13.75±3.72 % ID/g at 2 h, 4 h and 24 h post-injection. Lutetium-177 emits a 208 keV gamma ray that was directly imaged 2 hr postinjection. Radioactivity was clearly visible in the tumor as well as in the kidneys, with the remainder of the mouse body showing background levels of radioactivity.

The therapeutic efficacy of $^{177}$Lu-DOTA-Re(Arg$^{11}$) CCMSH was examined in B16/F1 melanoma-bearing mice [89]. Dark palpable tumors were identifiable 3 days post tumor cell implantation. Treatment groups of 10 mice were administered a saline placebo, 37.0 MBq, 2 × 18.5 MBq of $^{177}$Lu-DOTA-Re(Arg$^{11}$)CCMSH via tail vein injection. The second 18.5 MBq dose of $^{177}$Lu-DOTA-Re(Arg$^{11}$)CCMSH was given 4 days after the initial dose. In contrast to the placebo control group, the tumor growth rates of mice treated with 37.0 MBq and 2 × 18.5 MBq of $^{177}$Lu-DOTA-Re(Arg$^{11}$)CCMSH were substantially reduced. The placebo control group exhibited a 13.3±2.3 day mean survival time compared to 16.2±3.6 days and 15.1±1.8 days for the 37.0 MBq and 2 × 18.5MBq treatment groups. Improvements in mean survival times for the treatment groups compared to the saline placebo controls were significant ($P < 0.05$). The mean survival results also demonstrated that the fractionated 2 × 18.5 MBq dose administration was as effective as a single 37.0 MBq dose. Hematology profiles of the 37.0 MBq-treated group and control group showed a significant decrease in red blood cell and white blood cell counts 2 weeks after treatment. Platelet counts reached a nadir 1 week post-treatment. The treatment group receiving 2 × 18.5 MBq of $^{177}$Lu-DOTARe(Arg$^{11}$)CCMSH showed only a drop in white blood cells 2 weeks post-injection. Both treatment groups exhibited normal kidney function with no increases in serum creatinine levels observed. Postmortem histopathology results showed no evidence of radiation-related damage to the kidneys of the treatment groups.

The treatment of malignant melanoma will be focused on metastatic disease, since primary melanoma tumors are surgically removed. Metastases are likely to consist of disseminated small tumors. Radionuclides that yield short path-length high LET radiations appear to be well suited to treat metastases [78]. DOTA-Re(Arg$^{11}$)CCMSH peptide was labeled with $^{212}$Pb [82], the parent radionuclide of $^{212}$Bi, which yields an alpha particle and beta particles upon its decay [90]. The major advantage of targeting $^{212}$Pb ($t_{1/2}$ = 10.6 h) to the

tumor instead of $^{212}$Bi ($t_{1/2}$ = 60.6 min) is that $^{212}$Pb delivers greater than 10 times the dose per unit of administered activity compared to $^{212}$Bi alone or the alpha-emitter $^{212}$Bi [82]. Another benefit of administering $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH is that the radiolabeled peptide will circulate, target melanoma tumor cells and be cleared from the body as the $^{212}$Pb-labeled peptide and not the alpha-emitting $^{212}$Bi compound, minimizing normal tissue exposures. Peptide-targeted $^{212}$Pb, internalized and retained by tumor cells will decay to the alpha-particle emitting $^{212}$Bi, localizing the highly toxic short-ranged alpha radiation within the tumor. Finally, the 10.6 h half-life of $^{212}$Pb makes dose preparation and administration easier and than the short half-life $^{212}$Bi.

DOTA-Re(Arg$^{11}$)CCMSH was labeled with $^{212}$Pb eluted from a $^{224}$Ra/$^{212}$Pb radionuclide generator [91] (AlphaMed Inc, Acton, MA) and purified to homogeneity by reverse-phase HPLC [82]. Tumor uptake values were 11.25±1.52 % ID/g, 12.84±2.53 % ID/g and 4.59±1.45 % ID/g at 2 h, 4 h and 24 h post-injection. Whole-body clearance of radioactivity from normal organs and tissues was rapid with the exception of the kidney. Approximately 90% of the injected radioactivity was in the urine 2 h post-injection. Non-specific uptakes of radioactivity in the kidneys were 7.31±1.26 % ID/g, 4.56±1.27 % ID/g and 2.93±0.53 % ID/g at 2 h, 4 h and 24 h post-injection. The therapeutic efficacy of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH was determined in B16/F1 melanoma-bearing C57 mice [82]. Palpable dark tumors were detected 3 days post tumor cell inoculation. On the fourth day post-tumor cell implantation, groups of 10 mice were treated with 1.85 MBq, 3.7 MBq or 7.4 MBq of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH via tail vein injection, respectively. A control group of mice received a saline placebo injection. Tumor measurements and body weights were recorded daily. Mice receiving $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH treatment displayed dramatic reductions in tumor growth rates at all doses compared to the saline placebo control group. Twenty percent of the mice in the 3.7 MBq treatment groups and 45% of the mice in the 7.4 MBq group survived the entire 120-day therapy study. Postmortem histopathological examination of the tumor site and other major organs showed no sign of primary or metastatic melanoma or melanoma associated S100 antigen, allowing the mice to be classified as complete remissions or cures. The mean survival times for groups of mice receiving 1.85 MBq, 3.7 MBq and 7.4 MBq of $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH that did not survive the 120-day study were 22.0±5.5 days, 28.0±8.8 days and 49.8±27.3 days, respectively, compared to 14.6±4.4 days for the control group. Histopathological examination of the kidneys showed moderate toxicity at the 7.4 MBq dose as evidenced by damage to tubule and glomerular structures and a thin renal cortex, although no behavior affects were observed during the study. Kidney damage at the 1.85 MBq and 3.7 MBq doses was classified as minor.

Recently, a dual function therapy/diagnostic “theranostic” α-MSH analogue was reported. The $\alpha_v\beta_3$ integrin avid peptide sequence Arg-Gly-Asp (RGD) was conjugated to the (Arg$^{11}$)CCMSH α-MSH analogue via a lysine spacer [92]. It was reported that the RGD peptide could induce cell apoptosis by activating procaspase-3 directly upon entering the cell [93]. RGD-Lys-(Arg$^{11}$)CCMSH was radiolabeled and cyclized with $^{99m}$Tc [92]. $^{99m}$Tc-RGD-Lys-(Arg$^{11}$)CCMSH exhibited rapid high melanoma uptake of 14.83±2.94 %ID/g 2 h postinjection and prolonged tumor retention of 7.59±2.04 %ID/g 24 h postinjection in B16/F1 melanoma mouse model [92]. A single 3 h treatment of B16/F1 cultured melanoma cells with 100 nM of RGD-Lys-(Arg$^{11}$)CCMSH significantly ($p<0.05$) decreased their clonogenic survival by 65% compared to the untreated control cells [92]. The favorable melanoma targeting property of $^{99m}$Tc-RGD-Lys-(Arg$^{11}$)CCMSH and remarkable cytotoxic effect of RGD-Lys-(Arg$^{11}$)CCMSH in B16/F1 melanoma cells warrant further evaluation of $^{188}$Re-labeled α-MSH hybrid peptides for melanoma treatment.

## Clinical translation of radiolabeled MC1-R avid peptides for melanoma imaging and therapy

The incidence and mortality rates associated with melanoma have increased on the order of 3–7%/yr. during the past 10 years [94]. The current cumulative lifetime risk for melanoma is estimated to be approximately 1:75 in the US and as high as 1:25 in Australia. Annual mortality rates vary between 3–5 per 100,000 in the US to 5–10 per 100,000 in Australia [94]. Early melanoma tumor diagnosis and prompt surgical removal are a patient's best hope for a cure. Unfortunately, metastatic malignant melanoma is resistant to current chemotherapy and immunotherapy regimens. Survival rates for patients with lymph node metastases average 12–15 months whereas only 3–6% of patients with end-stage disease will survive 10 yrs. [95]. The niche for a melanoma selective imaging agent includes diagnosis of metastatic disease and monitoring melanoma tumor growth during and after rounds of chemotherapy or targeted radiotherapy. Novel melanoma selective imaging agents would make a significant impact in metastatic melanoma diagnosis and treatment management.

The underlying fundamentals for the clinical translation of MC1-R targeted radiolabeled peptide for melanoma imaging and therapy are strong, however, several challenge remain to be addressed. There is a large volume of preclinical work to support translation of MC1-R targeting radiolabeled α-MSH peptide analogues into the clinic for melanoma imaging and therapy. For example, over expression of the MC1-R on the surface of melanoma tumor cells is highly selective. The α-MSH analogues discussed in this review have nanomolar to subnanomolar affinity for the MC1-R [25–31] and are internalized upon MC1-R binding [96]. Peptide internalization is particularly important for radiotherapy, since it sequesters the radionuclide inside the tumor cell close to the nucleus. Toxcity and immunogenicity of the targeting peptide is not likely to be an issue. Since the early 60s α-MSH or α-MSH analogues have been injected intravenously into humans to induce tanning [23,97] or to over-come erectile dysfunction [98]. No toxic side effects were reported in any of these clinical applications. Radiolabeled α-MSH analogues will be injected at nanomolar concentrations, far below the concentration necessary to illicit a pharmacological response. The preclinical studies presented in this review demonstrate that α-MSH analogues, radiolabeled with SPECT and PET radionuclides, are able to image primary and metastatic melanoma tumors in mouse animals models. Likewise, the therapeutic efficacies of α-MSH analogues labeled with alpha and beta emitting radionuclide have been demonstrated in mouse melanoma models. Matching the decay properties of the therapeutic radionuclide with tumor size and tumor burden will further enhance treatment success. Imaging of the therapeutic radionuclide directly (i.e. $^{188}$Re, $^{177}$Lu) [87,89] or a matched pair surrogate (i.e. $^{203}$Pb for $^{212}$Pb) [99] will allow the determination of patient specific dosimetry and calculation of the treatment dose.

The major challenges to successful clinical translation of radiolabeled MC1-R avid peptides for melanoma imaging and therapy are high non-specific kidney uptake and low MC1-R receptor numbers per tumor cell. Clearance of low molecular weight compounds from the body, such as small peptides, is primarily through the kidneys. Negatively charged tubule membranes appear to be behind a large portion of the non-specific peptide renal retention. Accumulation of the targeting peptide labeled with a therapeutic radionuclide can lead to a significant radiation does to the kidney making it the dose limiting normal organ. Co-injection of positively charged Lys or dLys, amino acid mixtures, low molecular weigh albumin proteolytic fragments and plasma expanders can significantly reduce nonspecific kidney retention. Reduction of nonspecific kidney retention is critical for enhancing imaging and increasing the treatment dose permitted by the dose limiting organ.

The MC1-R has high specificity and affinity for the radiolabeled α-MSH analogs discussed in this review. Receptor mediated binding was only observed in tumor tissue, resulting in melanoma selective deposition of imaging and therapeutic radionuclides. The only drawback to targeting MC1-R for melanoma imaging and therapy is the fairly low number of receptors per melanoma tumor cell. Fewer receptors demand higher specific activity radiolabeled peptide preparations of high melanoma tumor uptake. That means efficient radiolabeling chemistry using very high specific activity radionuclide preparation for one step kit formulation of the radiopharmaceutical or a final step HPLC purification that can separate radiolabeled peptide from unlabeled peptide and any free radionuclide. The presence of non-radiolabeled peptide in the final imaging or therapeutic preparation will dramatically reduce if not eliminate melanoma tumor uptake.

In conclusion, the strong pre-clinical melanoma imaging and therapy data underscore the clinical potential use of radiolabeled α-MSH peptides. Successful translation of MC1-R avid melanoma imaging agents would have an important impact in disease diagnosis and treatment assessment. Radiotherapeutic peptides would bring a much needed new treatment option in an adjuvant setting. The aggressiveness and resistance to conventional treatment of end stage melanoma drives the development and testing of novel MC1-R avid tumor imaging and therapeutic radiolabeled peptides.

## Acknowledgments

The authors are also grateful for the imaging support from the VA Biomolecular Imaging Center at the Harry S Truman VA Hospital and the University of Missouri, and the Keck-UNM Small Animal Imaging Resource.

**Funding:** National Institutes of Health, NCI P50-CA103130, R42-CA85106 and 1R43CA11492, US Department of Energy ER61661, Southwest Melanoma SPORE Developmental Research Program, Oxnard Foundation, US Department of Defense W81XWH-09-1-0105 and National Institutes of Health NM-INBRE P20RR016480.

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**Table 1**

α-MSH peptide analog names, structures, molecular weights and $IC_{50}$ values.

| Name | Structure | MW | $IC_{50}$* nM |
|---|---|---|---|
| α-$MSH_{1-13}$ | Ac-[$Ser^{1}$-$Tyr^{2}$-$Ser^{3}$-$Met^{4}$-$Glu^{5}$-$His^{6}$-$Phe^{7}$-$Arg^{8}$-$Trp^{9}$-$Gly^{10}$-$Lys^{11}$-$Pro^{12}$-$Val^{13}$]-$NH_2$ | 1,665 | 1.19 |
| NDP | Ac-[$Nle^{4}$, D-$Phe^{7}$]-α-$MSH_{1-13}$ | 1,647 | 0.21 |
| DOTA-NDP | DOTA-[$Nle^{4}$, D-$Phe^{7}$]-α-$MSH_{1-13}$ | 1,992 | 0.22 |
| DOTA-$MSH_{OCT}$ | Ac-[βAla$^{3}$, $Nle^{4}$, D-$Phe^{7}$, $Lys^{10}$]-α-$MSH_{3-10}$ | 1,457 | 9.21 |
| DOTA-NAPamide | Ac-[$Asp^{5}$, $Nle^{4}$, D-$Phe^{7}$]-α-$MSH_{4-11}$ | 1,486 | 1.37 |
| DOTA-CCMSH | DOTA-[$Cys^{3}$, $Cys^{4}$, $Nle^{4}$, D-$Phe^{7}$, $Cys^{10}$]-α-$MSH_{3-13}$ | 1,792 | 4.9 |
| DOTA-ReCCMSH | DOTA-[$Cys^{3}$, $Cys^{4}$, $Nle^{4}$, D-$Phe^{7}$, $Cys^{10}$]-α-$MSH_{3-13}$ (Re) | 1,990 | 1.2 |
| DOTA-Re($Arg^{11}$)CCMSH | DOTA-[$Cys^{3}$, $Cys^{4}$, $Nle^{4}$, D-$Phe^{7}$, $Cys^{10}$, $Arg^{11}$]-α-$MSH_{3-13}$ (Re) | 2,018 | 2.1 |
| DOTA-CycMSH | DOTA-[$Lys^{2}$, $Nle^{4}$, D-$Phe^{7}$, $Arg^{11}$, $Asp^{14}$]-α-$MSH_{2-14}$ | 1,907 | 1.8 |
| DOTA-Gly-Glu-CycMSH | DOTA-Gly-Glu-[$Lys^{2}$, $Nle^{4}$, D-$Phe^{7}$, $Arg^{11}$, $Asp^{14}$]-α-$MSH_{2-14}$ | 2,093 | 0.9 |
| CBTE2A-Re($Arg^{11}$)CCMSH | CBTE2A-[$Cys^{3}$, $Cys^{4}$, $Nle^{4}$, D-$Phe^{7}$, $Cys^{10}$, $Arg^{11}$]-α-$MSH_{3-13}$ (Re) | 1,957 | 5.4 |
| D-$Lys^{2}$-Re($Arg^{11}$)CCMSH | Ac-[$Lys^{2}$,$Cys^{3}$, $Cys^{4}$, $Nle^{4}$, D-$Phe^{7}$, $Cys^{10}$, $Arg^{11}$]-α-$MSH_{3-13}$ (Re) | 1,804 | 5.4 |
| RGD-$Lys^{2}$-($Arg^{11}$)CCMSH | Arg-Gly-Asp-D-Tyr-Asp-[$Lys^{2}$, $Cys^{3}$, $Cys^{4}$, $Nle^{4}$, D-$Phe^{7}$, $Cys^{10}$, $Arg^{11}$]-α-$MSH_{2-13}$ | 2,150 | 2.1 |

*$IC_{50}$ values were determined by in vitro cell binding assays. NDP [28], DOTA-$MSH_{OCT}$ [29], DOTA-NAPamide [30], DOTA-CCMSH, DOTA-ReCCMSH [28], DOTA-Re($Arg^{11}$)CCMSH [32], DOTA-CycMSH, DOTA-Gly-Glu-CycMSH [33,34], D-$Lys^{2}$-Re($Arg^{11}$)CCMSH [35], RGD-$Lys^{2}$-($Arg^{11}$)CCMSH [37].