DATE: NOVEMBER 22, 2005

TO: Robert L Kladiva
US Department of Energy
Chicago Office
9800 South Cass Avenue
Argonne, IL 60439

FROM: Thomas P. Quinn
University of Missouri

SUBJECT: Final Report DE-FG02 93ER61661

Please find enclosed a paper copy and an electronic copy (CD) of the final report for DE-FG02 93ER61661. Please contact me if additional information is required. The financial documents were sent under a separate cover .

Sincerely

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DOE F 241.3
(2-01) p. 1 of 4

ANNOUNCEMENT

UNITED STATES DEPARTMENT OF ENERGY (DOE)
Scientific and Technical Information (STI) For Financial Assistance Recipients and Non-M&O/M&I Contractors

OMB Control No. 1910-1400

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DE FG02 93ER61661
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The University of Missouri, Columbia, MO

C. STI Product Title
Melanoma Therapy with Rhenium Cyclized Alpha Melanocyte Stimulating Hormone Peptide Analogs

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E. STI Product Issue Date/Date of Publication
11/21/2005 (mm/dd/yyyy)

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ANNOUNCEMENT

UNITED STATES DEPARTMENT OF ENERGY (DOE)
Scientific and Technical Information (STI) For Financial Assistance Recipients and Non-M&O/M&I Contractors

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Final Report: DOE DE FG02 93ER 61661

Abstract:
Malignant melanoma is the 6th most commonly diagnosed cancer with increasing incidence in the United States. It is estimated that 54,200 cases of malignant melanoma will be newly diagnosed and 7,600 cases of death will occur in the United States in the year 2003 (1). At the present time, more than 1.3% of Americans will develop malignant melanoma during their lifetime (2). The average survival for patients with metastatic melanoma is about 6-9 months (3). Moreover, metastatic melanoma deposits are resistant to conventional chemotherapy and external beam radiation therapy (3). Systematic chemotherapy is the primary therapeutic approach to treat patients with metastatic melanoma. Dacarbazine is the only single chemotherapy agent approved by FDA for metastatic melanoma treatment (5). However, the response rate to Dacarbazine is only approximately 20% (6). Therefore, there is a great need to develop novel treatment approaches for metastatic melanoma. The global goal of this research program is the rational design, characterization and validation of melanoma imaging and therapeutic radiopharmaceuticals. Significant progress has been made in the design and characterization of metal-cyclized radiolabeled alpha-melanocyte stimulating hormone peptides. Therapy studies with ^{188}Re-CCMSH demonstrated the therapeutic efficacy of the receptor-targeted treatment in murine and human melanoma bearing mice (previous progress report). Dosimetry calculations, based on biodistribution data, indicated that a significant dose was delivered to the tumor. However, ^{188}Re is a very energetic beta-particle emitter. The longer-range beta-particles theoretically would be better for larger tumors. In the treatment of melanoma, the larger primary tumor is usually surgically removed leaving metastatic disease as the focus of targeted radiotherapy. Isotopes with lower beta-energies and/or shorter particle lengths should be better suited for targeting metastases. The ^{177}Lu-DOTA-Re(Arg11)CCMSH and ^{212}Pb-DOTA-Re(Arg11)CCMSH complexes were developed and synthesized to investigate its ability to target and deliver an effective dose to small melanoma tumors and metastatic deposits. Dosimetry calculations for ^{188}Re-CCMSH and ^{212}Pb/^{212}Bi[DOTA]-Re(Arg11)CCMSH were compared in the B16/F1 mouse melanoma flank tumor model to analyze the delivered dose to tumor and normal organs.

A synopsis of the major accomplishments over the funding period

- Successful synthesis and characterization of DOTA conjugated ReCCMSH analogs that exhibit high melanoma tumor uptake and retention and rapid whole body clearance. DOTA-ReCCMSH analogs were radiolabeled with a number of beta and alpha particle emitting isotopes including ^{177}Lu, ^{149}Pm and ^{212}Bi which posses therapeutic decay properties.
- The Maximum tolerable dose of ^{188}Re-CCMSH(Arg^{11}) is greater than 1000 μCi/mouse. Studies showed no adverse physiological or histological side effects in mice receiving up to 1000 μCi of ^{188}Re-CCMSH(Arg^{11}).
- Therapy studies with ^{188}Re-CCMSH(Arg^{11}) demonstrated delayed tumor growth with increasing dose in both murine and human melanoma bearing mouse model systems. One human melanoma bearing scid mouse was "cured" in the 600 μCi/mouse trial. Higher dose mouse therapy trials at 1 mCi/mouse did not yield better therapy outcomes.
- Therapy studies with ^{177}Lu-DOTA-Re(Arg11)CCMSH demonstrated that with small melanoma tumors ^{177}Lu-DOTA-Re(Arg11)CCMSH treated animals showed no survival advantage over placebo treated animals. B16/F1 melanoma bearing animals were treated with up to 1 mCi of radioactivity. The lower energy beta emitter was not better than the high energy beta emitter Re-188 in small melanoma tumors.
- Therapy studies were performed with ^{212}Pb/^{212}Bi[DOTA]-Re(Arg11)CCMSH in B16/F1 bearing melanoma animals. The mice were treated with 50, 100 and 200 microcuries of ^{212}Pb/^{212}Bi[DOTA]-Re(Arg11)CCMSH. All the treated mice showed exhibited extended mean

survival times. Twenty percent of the 100 microcurie group and 45% of the 200 microcurie group survived the entire study and were melanoma free. Peptide targeted alpha-therapy was clearly most effective against small melanoma tumors than either of the beta-emitting radionuclides Re-188 or Lu-177. Since patients with end stage have disseminated metastases, short range high linear energy transfer alpha-particle emitting radionuclides like Bi-212 should be very effective.

Detailed Report:

Melanoma Therapy: The first task was to improve the synthetic yield of ^{188}ReCCMSH prior to beginning MTD and therapy studies. Initially, the yield of ^{188}ReCCMSH was approximately 10%. The low yields were primarily due to oxidation of the free thiols in the CCMSH peptide prior to complexation with ^{188}Re. The problem was eliminated by keeping our peptide preparations protected until they were ready to be used. Aliquots of CCMSH can be deprotected and prepared for labeling in 48 h. The deprotected peptide is stable in a lyophilized form for approximately three weeks. Using freshly deprotected and properly stored peptide, our yields of ^{188}ReCCMSH synthesis have jumped from 10% to 50% at the 50 mCi level. The amount of pure radioactive ^{188}ReCCMSH complex necessary for therapy studies is approximately 10 mCi. At the 10 mCi scale, the purified ^{188}ReCCMSH product showed instability. Two hours post purification, the ^{188}ReCCMSH preparation contained approximately 30 % perrhenate. Injection of this test complex into tumor bearing C57 mice showed tumor uptake (10.45 ±1.77 % ID/g) but also showed high activity in the stomach (18.21±3.44 ID/g) indicative of the free perrhenate. Based on the HPLC traces of the ^{188}ReCCMSH complex at various time points post synthesis, it appeared that the decomposition was likely due to reoxidation of the rhenium as opposed to radiolysis. To combat the unfavorable redox reaction, ascorbic acid was added to the complex synthesis reactions and to the purified sample immediately after purification. It was determined that the addition of 25 mg of fresh ascorbic acid to the reaction mixture and purified sample or to the purified sample alone resulted in dramatically improved complex stability. HPLC analysis of ^{188}ReCCMSH at 10 mCi/ml in the presence of 25 mg of ascorbic acid showed that the complex was stable 2, 4 and 24 h post purification. For example. *in vivo* the complex showed high tumor uptake (9.78±2.00 % ID/g) and greatly reduced stomach activity values (1.93±0.39 % ID/g).

Maximum tolerated dose studies were completed prior to initiating melanoma therapy studies in mice. Groups of 5 mice were injected with 100-600 uCi of ^{188}ReCCMSH per animal. A group of saline injected mice were used as control animals. Results for the 100 and 200 uCi per animals showed no adverse effects from radioactivity administration. No changes in white blood cell or platelet counts were observed in the mice receiving 100 and 200 uCi of ^{88}ReCCMSH. Mice treated with 400 and 600 uCi of ^{188}ReCCMSH per animal showed similar serum profiles and behavior as the 100 and 200 uCi groups. Mice receiving either 400 or 600 uCi of ^{188}ReCCMSH exhibited weight gain and showed vigorous activity levels similar to control animals over the entire 7-week study. There was no effect on red and white blood cell levels in any of the mice receiving the higher doses of ^{188}ReCCMSH. There was a slight decrease in white blood cell counts in the control mice on day two of the study but not in either group receiving ^{188}ReCCMSH. The ranges of platelet levels from week 1 through 7 were, control-mice 773 to 465, 400 uCi-mice 753 to 503 and the 600 uCi-mice 688 to 471. The highs and lows were reached in the control and treatment groups during the same time point. The greatest depression in platelet counts came 5 weeks after injection in both control and treated animals. After the seven-week MTD study the animals were sacrificed and the major organs sent for a post mortem histopathology examination. Results of both the gross pathology report and the histology report on the major organs revealed no differences between treated and control groups. Since the MTD studies on 188Re-CCMSH up to 600 uCi per mouse resulted in no observable side effects, therapeutic studies were initialed within the 100-600 uCi range.

Initial therapeutic studies were performed in B16 murine melanoma bearing C57 mice using the second-generation analog ^{188}ReCCMSH (Arg^{11}) (Figure 1). Typical therapy trial groups contained groups of 10 mice including, normal mice, tumor-mice with no treatment, tumor mice receiving a single dose therapy or tumor mice receiving multi-dose therapy. Single dose therapy was performed with 200 uCi and 600 uCi injections of ^{188}ReCCMSH (Arg^{11}). A multi-dose therapy trial was also performed with 2 x 400 uCi of ^{188}ReCCMSH (Arg^{11}). Tumor mice were injected with 1 million B16 cells in the flank. Four days after injection of the B16 cells small palpable dark melanoma tumors appeared. All mice in the study were weighted and time zero blood samples were obtained prior to injection of ^{188}ReCCMSH (Arg^{11}) or

the saline placebo through the tail vein. Tumor growth was retarded in the mice receiving the ^{188}ReCCMSH (Arg^{11}) injections, with greater regression in growth seen at the higher dose. Representative tumor sizes at day 3 pi were 0.05 cm^3, 0.04 cm^3 and 0.02 cm^3 for the control, 200 uCi and 600 uCi groups, respectively. By day 7 the tumor sizes were 0.27 cm^3, 0.23 cm^3 and 0.17 cm^3 for the control, 200 uCi and 600 uCi groups. A greater retardation of tumor growth was obtained with the 2 x 400 uCi multi-dose therapy trial. Animals received a 400 uCi injection on day 0 and a second dose on day 3. The tumor sizes were 0.03 cm^3 on day 3 and 0.15 cm^3 at day seven. None of the animal showed adverse side effects from the radiation doses. Red and white cell counts as well as platelet counts were similar between the control non-tumor bearing mice and the tumor mice receiving ^{188}ReCCMSH (Arg^{11}).

While administration of ^{188}ReCCMSH (Arg^{11}) caused measurable delays in tumor grow, none of the mice exhibited cures. B16 melanoma tumors are very aggressive and invasive. Mice injected with 1 million B16 cells subcutaneously generally survive 12-14 days. ^{188}ReCCMSH (Arg^{11}) therapy did increase the mean life expectancy of the B16 tumor bearing mice. The mean life expectancy for the untreated control group was 13.6±1.3 days with the 200 μCi, 600 μCi and 2x400 μCi treatment groups exhibiting 14.2±1.0 day, 14.3±1.3 day and 17.3±1.9 day expectancies, respectively. A significant increase in survivability was observed for the 2x400 μCi treatment group but not in the single dose groups. The results from this initial therapy study indicated that multi-dose therapy was more effective than high activity single dose therapy. These results will be incorporated into our experimental design for the next therapy studies performed in human melanoma bearing scid mice.

Therapy trials were also performed in TXM-13 human melanoma bearing Scid mice. Typical therapy trial groups contained groups of 10 mice including, normal mice, tumor-mice with no treatment, tumor mice receiving a single dose therapy or tumor mice receiving multi-dose therapy. Single dose therapy was performed at 600 μCi per animal while a multi-dose therapy trial was performed at 2 x 400 uCi Tumor mice were injected with 1 million B16 cells in the flank. All mice in the study were weighted and time zero blood samples were obtained prior to injection of ^{188}Re-CCMSH(Arg^{11}) or the saline placebo through the tail vein.

Tumor growth was retarded in the mice receiving the ^{188}Re-CCMSH(Arg^{11}) injections, with greater inhibition observed in the single dose600 μCi group. For example the average tumor sizes for the control non treated group and the 2x400 μCi and 600 μCi treatment groups 18 days after therapy were 0.263 cm^3, 0.160 cm^3 and 0.061 cm^3, respectively. Delays in tumor growth translated into increased mean life expectancies. None of the animal showed adverse side effects from the ^{188}Re-CCMSH(Arg^{11}) radiation doses. Red and white cell counts as well as platelet counts were similar between the control non-tumor bearing mice and the tumor mice (data not shown). The mean life expectancy for the non treatment control group was 39.6±15.0, with the 600 μCi and 2x400 μCi groups displaying mean life expectancies

Table 1: Biodistribution comparison among ^{111}In-labeled DOTA-ReCCMSH (ReCCMSH), DOTA-CCMSH (CCMSH), DOTA-CMSH (CMSH), and DOTA-NDP (NDP) in B16/F1 murine melanoma bearing C57 mice at 0.5, 2, 4 and 24 h p.i. (% ID/g) (n = 5) (mean ± SD).

		Tumor	Blood	Muscle	Heart	Lung	Liver	Kidney
0.5 h	ReCCMSH	10.4 ± 1.75	1.57 ± 0.37	0.42 ± 0.17	0.72 ± 0.21	1.70 ± 0.55	0.59 ± 0.15	13.0 ± 3.21
	CCMSH	7.26 ± 2.00*	9.37 ± 0.93***	0.49 ± 0.14	2.13 ± 0.29***	5.12 ± 1.65***	2.85 ± 0.33***	73.4± 12.1***
	CMSH	8.46 ± 1.48	1.89 ± 0.19	0.29 ± 0.11	0.54 ± 0.12	0.93 ± 0.14	0.69 ± 0.09	34.1± 4.4***
	NDP	8.31 ± 2.02	0.59 ± 0.06**	4.49 ± 0.47***	0.43 ± 0.20*	0.67 ± 0.20***	0.58 ± 0.06	8.96± 2.24*
2 h	ReCCMSH	11.4 ± 2.89	0.07 ± 0.03	0.05 ± 0.03	0.07 ± 0.05	0.18 ± 0.07	0.23 ± 0.01	8.98 ± 0.82
	CCMSH	5.89 ± 1.88**	1.20 ± 0.32**	0.11 ± 0.04*	0.43 ± 0.14**	1.07 ± 0.22***	1.20 ± 0.31**	63.2 ± 15.6***
	CMSH	7.51 ± 0.98*	0.41 ± 0.10**	0.06 ± 0.03	0.07 ± 0.07	0.29 ± 0.06*	0.46 ± 0.04**	38.4 ± 3.6**
	NDP	8.62 ± 3.30	0.22 ± 0.08**	4.46 ± 0.72***	0.19 ± 0.10	0.74 ± 0.18**	0.54 ± 0.04***	12.0 ± 1.96*
4 h	ReCCMSH	9.49 ± 0.90	0.03 ± 0.03	0.09 ± 0.06	0.12 ± 0.10	0.11 ± 0.05	0.20 ± 0.04	9.27 ± 2.65
	CCMSH	4.32 ± 0.59***	0.82 ± 0.13***	0.17 ± 0.10	0.42 ± 0.09**	0.86 ± 0.34**	1.37 ± 0.47**	67.7 ± 8.1***
	CMSH	6.72 ± 1.21**	0.16 ± 0.06**	0.17 ± 0.10	0.18 ± 0.08	0.30 ± 0.24	0.44 ± 0.04**	37.0 ± 4.9***
	NDP	7.45 ± 2.38	0.11 ± 0.03**	3.32 ± 0.51***	0.30 ± 0.26	0.35 ± 0.14*	0.56 ± 0.11***	12.9 ± 2.49
24 h	ReCCMSH	4.86 ± 1.52	0.01 ± 0.01	0.03 ± 0.01	0.12 ± 0.11	0.06 ± 0.04	0.15 ± 0.02	5.64 ± 1.31
	CCMSH	1.91 ± 0.56**	0.08 ± 0.02**	0.04 ± 0.02	0.14 ± 0.04	0.15 ± 0.05*	0.72 ± 0.03***	45.5 ± 5.1***
	CMSH	3.09 ± 0.32*	0.02 ± 0.01**	0.02 ± 0.01	0.08 ± 0.08	0.12 ± 0.10	0.28 ± 0.05**	21.8 ± 1.7***
	NDP	2.47 ± 0.79*	0.02 ± 0.02	0.73 ± 0.22**	0.11 ± 0.08	0.25 ± 0.18	0.38 ± 0.11*	10.2 ± 2.72*

- $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; $P < 0.001$

To determine if Re-cyclization was necessary for high tumor uptake values and favorable pharmacokinetics of DOTA-ReCCMSH, several closely related non-Re cyclized analogs were synthesized for comparison. Compared with its linear counterpart ^{111}In-DOTA-CCMSH complex, the cyclized ^{111}In-DOTA-ReCCMSH complex exhibited a significant increase *in vivo* tumor targeting capacity. Uptake values for the Re-cyclized ^{111}In -DOTA-ReCCMSH complex and linear ^{111}In -DOTA-CCMSH complex were 9.49±0.90 and 4.32±0.59 % ID/g 4 h post injection. The tumor uptake value for the Re-cyclized complex (9.49±0.90 % ID/g) was also significantly higher that the disulfide bond-cyclized complex (6.72±1.21 % ID/g), indicating that the two methods of peptide cyclization were not equivalent. ^{111}In DOTA-NDP tumor uptake was also lower (7.45±2.38 % ID/g), without significance, than that of ^{111}In -DOTA-ReCCMSH (9.49±0.90 % ID/g), although it exhibited an unexpectedly high accumulation in the muscle (3.32±0.51 % ID/g). These results clearly showed that Re-cyclization was critical for high tumor uptake and retention as well as rapid clearance from normal tissues.

An comparison of the kidney uptake values for the ^{111}In -DOTA-MSH complexes at 2 h post injection showed that ^{111}In -DOTA-ReCCMSH had the lowest retention value. The kidney activity levels for ^{111}In -DOTA-ReCCMSH, ^{111}In -DOTA-CCMSH, ^{111}In -DOTA-CMSH, and ^{111}In DOTA-NDP were 8.98±0.82, 63.2±15.6, 38.4±3.6, and 12.0±1.96 % ID/g, respectively. Re cyclization appears to be responsible for the superior whole-body clearance properties of ^{111}In DOTA-ReCCMSH compared to the other closely related analogs. The analogs with free thiols or a disulfide bond have significantly higher kidney retention values than their Re-cyclized counter part.

Finally, we focused on reducing the non-specific kidney activity associated with ^{188}Re-CCMSH and ^{111}In -DOTA-ReCCMSH. The dose limiting normal tissue in our therapy studies will be the kidney, since approximately 95% of the injected dose clears through this organ. The initial kidney activities of ^{188}Re-CCMSH and ^{111}In -DOTA-ReCCMSH 30 min post injection were 16.08±3.58 and 13.0±3.21 % ID/g, respectively. Both Re-cyclized analogs showed superior kidney clearance properties to the 99mTc-cyclized homolog ^{99m}TcCCMSH (20.6±2.4 % ID/g) at 30 min post injection. The ability of amino acids or amino acid derivatives to further reduce non-specific kidney retention was systematically

investigated. The effects of ^{111}In -DOTA-ReCCMSH co-injection with lysine (30 mg) or ip injection of poly-lysine (1mg), cysteine (15 mg), cysteine dimethylester (10 mg), and dimercaptosuccinate (5 mg) 30 min prior to ^{111}In -DOTA-ReCCMSH injection were investigated in normal mice. Kidney uptakes for the lysine, polylysine, cysteine dimethylester and dimercaptosuccinate 2 h post injection were 4.30±0.30, 5.71±0.50, 5.51±0.53, 7.13±1.18, and 9.58±1.50 % ID/g, respectively. These results showed that co-injection of lysine·HCl was capable of reducing kidney uptake by another 50% to 4.30±0.30 % ID/g. Free cysteine was equally effective in reducing kidney uptake as polylysine. Cysteine dimethylester and dimercaptosuccinate injection did not show any kidney protective properties. This result was very interesting in light of the biodistribution results presented for the non Re-cyclized ^{111}In -DOTA-CCMSH molecule. It appears that free sulfhydryls contribute significantly to non-specific kidney uptake. IP injection of free cysteine prior to radiolabeled peptide injection appears to have a protective effect on the kidney. It is evident from these results that lysine co-injection will be important in reducing non-specific kidney damage during our therapy studies. A cocktail of lysine and cysteine may be even more effective in reducing kidney retention of our radiolabeled ReCCMSH analogs.

Table 2: MSH analogs used in studies to improve tumor uptake and reduce kidney retention.

ReCCMSH	Ac-Cys-Cys-Glu-His-D-Phe-Arg-Trp-Cys-Lys-Pro-Val-NH_2
DOTA-ReCCMSH	**DOTA**-Cys-Cys-Glu-His-D-Phe-Arg-Trp-Cys-Lys-Pro-Val-NH_2
Ac-Lys(DOTA)-ReCCMSH	Ac-**(Lys-DOTA)**-Cys-Cys-glu-His-D-Phe-Arg-Trp-Cys-Lys-Pro-Val-NH_2
DOTA-ReCCMSH(Arg11)	**DOTA**-Cys-Cys-Glu-His-D-Phe-Arg-Trp-Cys-**Arg**-Pro-Val-NH_2
DOTA-ReCCMSH-OH	**DOTA** -Cys-Cys-Glu-His-D-Phe-Arg-Trp-Cys-Lys-Pro-Val-**OH**
DOTA-ReCCMSH-Asp14-OH	**DOTA** -Cys-Cys-Glu-His-D-Phe-Arg-Trp-Cys-Lys-Pro-Val-**Asp-OH**
NDP	Ac-Ser-Tyr-Ser-Met-Glu-His-D-Phe-Arg-Trp-Cys-Lys-Pro-Val-NH_2 .

ReCCMSH Analog Refinement: Chemical characterization of [D-Lys]-ReCCMSH and DOTA-ReCCMSH were performed to develop ReCCMSH analogs with greater radiolabeling versatility and improved tumor to normal organ ratios. Several DOTA-ReCCMSH analogs, Ac-Lys(DOTA)-ReCCMSH, DOTA-ReCCMSH(Arg^{11}), DOTA-ReCCMSH-OH, and DOTA-ReCCMSH-Asp14-OH were synthesized using solid phase peptide synthesis (SPPS) followed by rhenium cyclization. The biodistribution of [DOTA-D-Lys]-ReCCMSH was compared to [DOTA]-ReCCMSH to see if the location and/or chemical linkage of DOTA affected the biodistribution patterns of the molecules. The IC-50 values of the metallopeptides were determined through competitive binding assays against (I-125-Tyr-2)-NDP. Radiolabeling of the DOTA-rhenium cyclized peptides with ^{111}In was carried out in NH_4OAc (0.1 M; pH 5.5) buffered solution for 30 min at 70 degrees C. The stability of radiolabeled complexes was demonstrated in 0.01 M pH 7.4 PBS/0.1% BSA. After separation of the radiolabeled peptide from the unlabeled peptide by RP-HPLC, the biodistribution of the radiolabeled complexes was performed in C57 mice bearing B16/F1 murine melanoma tumors. All radiolabeled complexes showed fast blood clearance (2 h p.i. ^{111}In- DOTA-ReCCMSH, 0.07±0.03%ID/g, Ac-Lys(^{111}In -DOTA)-ReCCMSH 0.09±0.06%ID/g, ^{111}In -DOTA-ReCCMSH(Arg^{11}) 0.21±0.08%ID/g), and their clearance was predominantly through the urine (4 h p.i. 93.5±1.7%, 87.8±6.5%, and 89.8±4.2% (%ID) for ^{111}In labeled DOTA-ReCCMSH, Ac-Lys(DOTA)-ReCCMSH, and DOTA-ReCCMSH(Arg^{11}), respectively. Tumor uptake values of 9.45±0.90, 6.01±2.36, and 17.41±5.61 (%ID/g) for ^{111}In labeled DOTA-ReCCMSH, Ac-Lys(DOTA)-ReCCMSH, and DOTA-ReCCMSH(Arg^{11}), at 4 h p.i., were observed, respectively. The kidney uptake was 9.27±2.65 %ID/g for In-111-DOTA-ReCCMSH, 19.02±2.63 %ID/g for Ac-Lys(^{111}In -DOTA)-ReCCMSH and 7.37±1.13 %ID/g for ^{111}In -DOTA-ReCCMSH(Arg^{11}) at 4 h post imjection. ^{111}In -DOTA-ReCCMSH(Arg^{11}) showed high melanoma uptake and lower kidney uptake than the corresponding Lys11 analogs, supporting the theory that lysine itself contributes to appreciable kidney uptake of DOTA-ReCCMSH. Neither DOTA-ReCCMSH-OH, nor DOTA-ReCCMSH-Asp-OH showed superior tumor/kidney ratios to DOTA-ReCCMSH or Ac-Lys(DOTA)-

ReCCMSH. The presence of more ionizable groups and an overall net negative charge did not improve kidney clearance over the Lys-11 to Arg substitution.

Based on the improved tumor uptake properties and lower kidney retention characteristics of the DOTA conjugated Ac-Lys-ReCCMSH(Arg^{11}) analog, the original ReCCMSH sequence was altered to contain arginine, at position 11 instead of lysine. The goal was to determine the optimal amino acid at this position. Also, biodistribution data from ReCCMSH and its analogs suggested that the kidneys are likely to be a dose-limiting organ. Therefore, lysine, arginine and a mixture of lysine and arginine were co-injected with radiolabeled ReCCMSH complexes to determine their efficacy in reducing activity in the kidneys. The phamacokinetics of ^{188}Re-CCMSH and ^{188}Re-CCMSH(Arg^{11}) were determined in B16/F1 murine melanoma bearing C57 mice. The tumor uptake values of ^{188}Re -CCMSH and ^{188}Re -CCMSH(Arg^{11}) were 15.03±5.20% ID/g and 20.44±1.91% ID/g at 1 hr post-injection and 1.94±0.47% ID/g and 3.50±2.32% ID/g at 24 hr post injection. Renal retention of ^{188}Re-CCMSH(Arg^{11}) was 11.79±1.29 ID/g and 3.67±051 ID/g at 1 hr and 4 hr post injection, which was a greater than 50% reduction compared with ^{188}Re-CCMSH. The Arg for Lys substitution in ^{188}Re-CCMSH(Arg^{11}) resulted in improved tumor uptake and retention properties coupled with reduced kidney retention. Co-injection of *L*-lysine, *D*-lysine *L*-arginine, and a combination of *L*-lysine and *L*-arginine were also investigated for their abilities to improve the tumor to kidney uptake ratios reducing of the radiolabeled complexes. Renal retention of both ^{188}Re-CCMSH and ^{188}Re-CCMSH(Arg^{11}) were significantly reduced by co-injection of twenty milligrams of *L*-lysine, *L*-arginine, and a combination of *L*-lysine and *L*-arginine. Tumor to kidney rations for ^{188}Re-CCMSH and ^{188}Re-CCMSH(Arg^{11}) were reduced by 46% and 32%, respectively. However, even with amino acid co-injection, the tumor/kidney ratio of ^{188}Re-CCMSH was lower than that of ^{188}Re-CCMSH(Arg^{11}). The improved tumor uptake and reduced kidney retention of ^{188}Re-CCMSH(Arg^{11}) will facilitate targeted irradiation of melanoma tumors while minimizing the dose to the kidneys.

Lu-177[DOTA]-ReCCMSH(Arg^{11}) biodistribution studies: Initial ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) biodistribution studies were performed with in B16/F1 murine melanoma bearing mice. [DOTA]-ReCCMSH(Arg^{11}) was labeled with ^{177}Lu (Univ. Missouri Research Reactor, MURR). For biodistribution studies, 4 μCi of HPLC purified ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) was injected into tumor bearing C57 mice through the tail vein. The mice were sacrificed at various time points and the tumor and normal tissues of interest were collected, weighed and counted. Results from the ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) biodistribution study are shown in Table 4. The biodistribution results demonstrate that the ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) complex excellent tumor uptake and retention properties (Table 4A). Clearance through the normal tissues was rapid except for the kidneys, which displayed similar or greater activity as the tumor. Activity in the tumor could be effectively quenched by co-injection of the super potent α-MSH analog NDP, indicating that tumor uptake was receptor mediated (Table 4B). NDP co-injection did not affect activity levels in the kidney suggesting that the activity was not receptor mediated.

A	% ID/gram		
Tissues	1hr	4hr	24hr
Tumor	14.48±0.85	17.68±3.32	9.05±4.31
Brain	0.06±0.06	0.13±0.12	0.10±0.03
Blood	0.19±0.22	0.12±0.16	0.12±0.07
Heart	0.01±0.02	0.12±0.10	0.17±0.11
Lung	0.69±0.28	0.02±0.03	0.05±0.05
Liver	0.44±0.05	0.43±0.07	0.49±0.07
Spleen	0.22±0.26	0.12±0.13	0.10±0.00
Stomach	0.47±0.40	0.26±0.29	0.73±0.21
Kidneys	17.99±2.47	19.09±2.38	13.75±3.72
Muscle	0.20±0.23	0.21±0.15	0.04±0.03
Pancreas	0.19±0.22	0.30±0.30	0.02±0.02
Carcass	0.18±0.05	0.15±0.05	0.11±0.01
Bone	0.07±0.09	0.25±0.19	0.21±0.05
	% ID		
Intestine	0.42±0.15	0.79±0.51	0.39±0.08
Urine	91.69±0.57	92.20±1.21	93.44±0.91

B	% ID/gram 1 hr
Tissues	^{177}Lu-MSH+ NDP
Tumor	0.92±1.30
Brain	0.07±0.10
Blood	0.21±0.30
Heart	0.00±0.00
Lung	0.91±0.71
Liver	1.78±0.45
Spleen	1.80±0.01
Stomach	0.68±0.66
Kidneys	21.58±3.85
Muscle	0.05±0.07
Pancreas	0.46±0.64
Carcass	0.16±0.01
Bone	0.00±0.00
	% ID
Intestine	0.47±0.04
Urine	90.70±0.21

Table 4. **(A)** Biodistribution of ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) or **(B)** ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) (^{177}Lu-MSH) plus the super potent α-MSH analog NDP or L-lysine (25 mg) co-infusion in B16/F1 murine melanoma C57 mice (% ID/g and % ID, n=4).

The therapeutic efficacy of ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) was examined in the B16/F1 flank melanoma mouse model. Mice were treated with 500 microcuries and 1000 microcuries of ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) or a saline placebo. Typical therapy trial groups contained groups of 10 mice including, normal mice, tumor-mice with no treatment, tumor mice receiving a single dose therapy. Tumor mice were injected with 1 million B16 cells in the flank. Four days after injection of the B16 cells small palpable dark melanoma tumors appeared. All mice in the study were weighted and time zero blood samples were obtained prior to injection of ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}). Mice were injected with ^{177}Lu[DOTA]-ReCCMSH(Arg^{11}) through the tail vein on day 1 (Figure 7).

All of the animals tolerated the doses with no signs of adverse effects from radiation. Mean survival times for the un treated controls was 13.3±2.3 days, while mice receiving 2x500 μCi exhibited a mean survival of 15.1±1.8 days and the 1000 μCi group 16.2±3.6 days. An examination of the tumor growth data showed that there was no statistical difference in tumor growth between the treatment groups and the placebo groups. Survival analysis also demonstrated no statistically significant improvement in mean life extension. Lu-177 therapy resulted in the lowest absorbed dose to the tumor per dosimetry calculations (see section III). This may explain the lack of therapeutic efficacy at the doses administered. It appears that one would have to double the administered dose to significant improvements in survival.

Biodistribution studies of 90Y-[DOTA]-Re(Arg11)CCMSH.

C57 BL/6 female mice, 7-8 weeks old, were inoculated subcutaneously in the right flank with one-million cultured B16/F1 murine melanoma cells. Ten days after the inoculation, when tumors reached a weight of approximately 500 mg, each mouse was injected with 2 uCi of 90Y-labeled peptide through the tail vein for in vivo studies. After radioactivity administration, the mice were housed separately and their urine and feces were collected. Groups of 5 mice were sacrificed at different time points of post injection. Tumors and normal tissues of interest were dissected, and the blood on the samples was sponged off with gauze. Contents in the gastrointestinal tract were not removed. The whole body and tissue samples were weighed and their radioactivity measured in a gamma counter. The total blood value

was counted as 6.5% of the whole body weight. The radioactivity uptake in the tumor and normal tissues of interest was expressed as a percentage of the injected radioactivity dose per gram of tissue (% ID/g) or percentage of the injected dose (% ID). All the animal studies were carried out in compliance with Federal and local Institutional rules for the conduct of animal experimentation. Statistical analysis was performed using the Students t-test for unpaired data. 90Y-[DOTA]-Re(Arg11)CCMSH exhibited fast cellular internalization and long cellular retention in B16/F1 cells. The tumor uptake of 90Y-[DOTA]-Re(Arg11)CCMSH was 25.70+/-4.64 %ID/g and 14.09+/-2.73 %ID/g at 2 and 4 hrs post-injection in B16/F1 melanoma bearing C57 mice (8). There was very little activity in blood and major organs such as liver, lung and muscle except for the kidney, which was main excretion organ for 90Y-[DOTA]-Re(Arg11)CCMSH The kidney uptake of 90Y-DOTA-Re(Arg11)CCMSH was 28.03+/-4.66 %ID/g and 24.86+/-4.89 %ID/g at 2 and 4 hrs post-injection in B16/F1 melanoma bearing C57 mice.

III. Dosimetry Studies.

Dosimetry calculations were performed by Darrell Fisher, Pacific Northwest National Laboratories for 188Re-(Arg11)CCMSH and 177Lu[DOTA]-Re(Arg11)CCMSH based on biodistribution studies performed in B16/F1 melanoma bearing mice. The absorbed dose to the B16/F1 melanoma tumor from 188Re-(Arg11)CCMSH was 3.022 cGy/uCi. The primary and secondary critical normal organs were large intestine and kidneys, with absorbed dose of 1.462 cGy/uCi and 0.685 cGy/uCi, respectively. The dosimetry calculations for 90Y-[DOTA]-Re(Arg11)CCMSH were 3.67 cGy/uCi to the tumor and 0.73 cGy/uCi to the intestine and 4.88 cGy/uCi to the kidneys. Overall, the dose from 90Y-[DOTA]-Re(Arg11)CCMSH was approximately 20% higher than 188Re-(Arg11)CCMSH. However, the kidney dose was approximately 8 times the dose deposited by 188Re-(Arg11)CCMSH. These results demonstrate that kidney protective measures will have to be employed if 90Y-[DOTA]-Re(Arg11)CCMSH is used for therapy. The lower energy beta emitter 177Lu had approximately half the deposited energy per administered microcurie of activity that 188Re or 90Y. The absorbed dose to the tumor was 1.48 cGy/uCi. The kidney dose was 0.428 cGy/uCi, which was in the same range as the high energy beta emitter 90Yand 188Re labeled peptide. There was greater non-specific retention of the 177Lu radiolabeled peptide than the 188Re or 90Y radiolabeled peptides resulting in the high absorbed dose to the kidneys. Finally, the dosimetry calculations for alpha-particle emitting were compared to their beta emitting counterparts. Calculated energy deposition from 212Pb/212Bi[DOTA]-Re(Arg11)CCMSH was 61.2 cGy/uCi to the tumor and 2.58 cGy/uCi to the intestine and 36.1 cGy/uCi to the kidneys. Overall, the dose from 212Pb/212Bi[DOTA]-Re(Arg11)CCMSH was 20 times higher per uCi than ^{188}Re-(Arg11)CCMSH or 90Y-[DOTA]-Re(Arg11)CCMSH. Preliminary therapy results appear to be consistent with the dosimetry findings in that tumor progression is greatly delayed or is reversed and cured in mice receiving 100 or 200 uCi 212Pb/212Bi[DOTA]-Re(Arg11)CCMSH. The 212Pb/212Bi[DOTA]-Re(Arg11)CCMSH therapy appears to be far more potent than 188Re-. 90Y-, or 177Lu- (Arg11)CCMSH in small but highly aggressive B16/F1 melanoma tumors.

In summary the dosimetry calculations fit the therapy results. The high LET alpha emitter yielded the highest therapy result followed by the low LET but high energy beta emitter 188Re and lastly the lower energy beta emitter 177Lu. We would predict the 90Y therapy would yield similar results to 188Re therapy, based on the dosimetry results. Our results support peptide targeted high energy beta-emitters like Re-188 and Y-90 for radiotherapy of large tumors or large tumor burdens while peptide targeted alpha-emitters would be the clear choice for smaller metastatic tumor deposits. Pb-212/Bi212 contains both an alpha-emission and a high energy beta providing both localized energy deposition coupled with larger area of homogeneous beta-radiation, treating a wide range of tumor sizes.

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