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Final Report:
Development and Testing of a $^{212}Pb/^{212}Bi$ Peptide for Targeting Metastatic Melanoma

CRADA PNNL/288, PLA1
(GIPP Project PNNL T2-291)

DR Fisher

October 2012

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Pacific Northwest National Laboratory
Richland, Washington 99352

PNNL Final Report
CRADA PNNL/288 (GIPP Project PNNL T2-291)

Development and Testing of $^{212}Pb/^{212}Bi$ Peptide for Targeting Metastatic Melanoma

Project Participants

V.G. Khlopin Radium Institute
Scientific Production, Department of Isotopes
St. Petersburg, Russia

AlphaMed, Inc.
Acton, Massachusetts; and Victoria, British Columbia

Pacific Northwest National Laboratory
Isotope Sciences Program
Richland, Washington

University of Missouri
Department of Biochemistry
Columbia, Missouri

Project Roles and Responsibilities

This project addressed several difficult technical issues and challenges, including radioisotope production, safe handling and transportation, protein synthesis, protein labeling, radiolabeled protein safety characterization and safety analysis, and pre-clinical biodistribution in laboratory animals. The team assembled, as described below for this project, provided each of the scientific and technical work elements needed for project success:

V.G. Khlopin Radium Institute, St. Petersburg, Russia conducted research in nuclear physical, radiochemical, geochemical, and ecological fields, associated with the problems of nuclear power engineering, radioecology, and isotope production and represents one of Russian and world-wide leaders in these directions. The Khlopin Radium Institute actively participates in federal programs and international projects related to nuclear physics, radiochemistry, radioecology, and radiogeochemistry. On this study, Khlopin prepared Pb-203 and performed peptide-labeling and purification studies. Khlopin also managed work involving the preparation and characterization imaging and biodistribution of Pb-203 and Pb-212-labeled peptides under the Project Partner Agreement.

AlphaMed, Inc. develops supplies of alpha-emitter isotopes for medical applications such as new cancer-treatment agents. AlphaMed performs work under contract with the Department of Energy and the National Cancer Institute. Much of this work is performed for AlphaMed at PNNL. On this project, AlphaMed provided scientific guidance and implementing background technology, oversaw all aspects of materials development and applications research, and directed the commercialization and business planning efforts.

Pacific Northwest National Laboratory supports the mission objectives of the Department of Energy, which include methods for improving the supply of isotopes for applications in science, medicine,

industry, and national security using the capabilities of its professional staff and the infrastructure associated with the Radiochemical Processing Laboratory. On this project, PNNL provided the lead-212/bismuth-212 generators for use on this project in Russia, and also provided internal radiation dosimetry calculations.

The University of Missouri at Columbia develops and tests new radiopharmaceuticals and conducts research associated with peptide labeling, biodistribution studies, and small-animal imaging. On this project, the University of Missouri provided scientific support and assistance to the Khlopin Radium Institute in peptide labeling under an authorized project subcontract from PNNL.

Introduction and Background

Metastatic melanoma (common skin cancer) develops from malignant transformation of the melanocyte, a cell that produces the pigment melanin. Melanomas spread superficially or systemically by nodular invasion. Metastases spread to regional lymph nodes and then distally to the lungs, liver, bone, and brain, where they grow into large tumor masses. Melanoma, a cell type that is highly radiation-resistant, is treated by wide surgical excision of primary tumors. However, distal symptomatic metastases can only be treated by chemotherapy (dicarbazine, temozolomide, paclitaxel, or cisplatin), which produce low response rates to therapy (15%), serious side-effects, and no improvement in overall survival. Current medical treatments are rarely successful, and the prognosis for patients diagnosed with metastatic melanoma is poor (Claveau et al., 2009). The need for improved and more effective therapies against metastatic melanoma is well-confirmed by recent clinical studies.

Radiation biologists recognize that alpha particles are effective in killing melanoma cancer cells. High-linear-energy alpha particles can be effective in treating radiation-resistant disease that responds poorly to traditional low-linear-energy beta/gamma emitters (Sgouros et al., 2010). Because of the short range of alpha particles (40 to 70 micrometers in soft tissue), and to be effective in cell killing, the alpha-emitters need to be delivered directly to cancer cells using cell-targeted radioimmunotherapy.

Miao et al. (2005) treated laboratory mice with rhenium-186- and lead-212-labeled-[DOTA]-Re(arg^{11})CCMSH, a novel class of metal-cyclized melanotropin peptide analogues for melanoma imaging and therapy that target the melanocortin-1 receptor on melanoma cells. Alpha-particles from bismuth-212, the first decay daughter of lead-212 complexed with DOTA]-Re(arg^{11})CCMSH were used to treat mice inoculated with melanoma cells. Miao et al. (2005) showed that treatment significantly decreased tumor growth, resulting in extended survival times and in many cases (45%), complete remission of disease. However, laboratory techniques must be further developed, tested, and verified for successful commercialization and Food and Drug Administration approval.

Purpose and Objectives

The purpose of this project is to develop a new radiolabeled peptide for imaging and treating metastatic melanoma. The immunoconjugate consists of a receptor-specific peptide that targets melanoma cells. The beta-emitter lead-212 (half-life = 10.4 hours) is linked by coordination chemistry to the peptide. After injection, the peptide targets melanoma receptors on the surfaces of melanoma cells. Lead-212 decays to the alpha-emitter bismuth-212 (half-life = 60 minutes). Alpha-particles that hit melanoma cell nuclei are likely to kill the melanoma cell. For cancer cell imaging, the lead-212 is replaced by lead-203 (half-life = 52 hours). Lead-203 emits 279 keV photons (80.1% abundance) that can be imaged and measured for biodistribution analysis, cancer imaging, and quantitative dosimetry.

Previous studies in mice showed that the radiolabeled peptide successfully treated metastatic melanoma in a high percentage of mice with metastatic melanoma, indicating excellent potential for treating human melanoma.

To develop and test the lead/bismuth-labeled peptide, the project was divided into two research phases. The first phase involved development of materials for imaging studies using the gamma-emitter lead-203 as a diagnostic substitute for lead-212. Phase I involved preparing lead-203, synthesizing the peptide, and conjugating the radionuclide with the peptide. Phase II involved preparing and testing the lead-212-labeled peptide. The lead-212/bismuth-212 generator system was prepared by the isotopes sciences program at Pacific Northwest National Laboratory (PNNL). Technical support was provided by AlphaMed, Inc., and by the University of Missouri (Columbia).

Lead-212 and bismuth-212 belong to a natural radioactive decay chain (Figure 1).

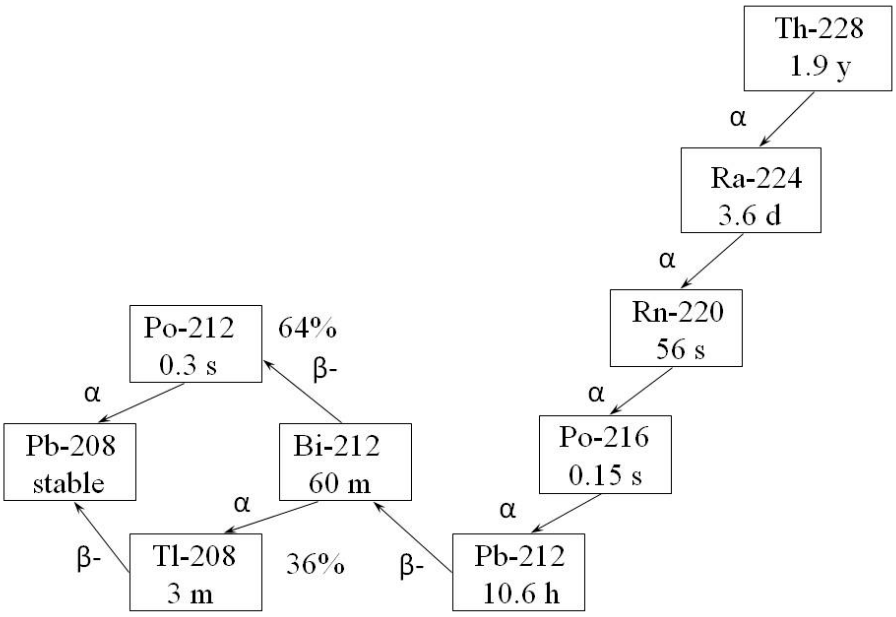

Figure 1. The thorium-228 natural decay chain.

Successful development of a radiolabeled peptide will lead to applications in cancer treatment. Drug development focuses on optimization of material characteristics and drug behavior in living systems.

Research Approach

In Phase I, Pacific Northwest National Laboratory and the Khlopin Radium Institute determined whether the gamma-emitter Pb-203 can be produced at the cyclotron facilities of KRI or whether it must be purchased commercially. Russian capabilities were tapped to synthesize the peptide and its conjugate to a ligand capable of binding the radioisotopes. The products of the syntheses were characterized and purified prior to further studies. The gamma-emitting radioisotope lead-203 was prepared and linked to the peptide conjugate. The radiolabeled construct was purified by chromatographic techniques and characterized by

standard radiochemical methods to prepare for follow-on biodistribution studies in normal and tumor-bearing animals. Results of such studies provide the technical basis for radiation dose calculations and other information that is needed for determining the appropriateness of a Bi-212-peptide for therapy of melanoma.

In Phase II, Pacific Northwest National Laboratory prepared and shipped the Pb-212/Bi-212 generator to the Khlopin Radium Institute in St. Petersburg, Russia. The studies performed in Phase I to prepare and evaluate Pb-203-labeled peptide were repeated using Pb-212. The Pb-212-peptide construct was purified by chromatographic techniques and characterized by standard radiochemical techniques.

The imaging studies at the Khlopin Radium Institute served as a platform for capitalizing on existing capabilities and further developing the following capabilities:

- production of Pb-203 at the Khlopin cyclotron facility
- development and refinement of technology for synthesizing and purifying the ligand-peptide (DOTA)-Re(Arg11)CCMSH
- preparation of Pb-203-labeled peptide
- synthesis, purification and characterization of the radiolabeled peptide

Production of Pb-203. The method of choice for producing Pb-203 is irradiation of a thallium-203 target in the Khlopin cyclotron to produce Pb-203 directly through a (p,n) reaction. The technology for separating elemental lead from thallium has been thoroughly developed for the production of Tl-201, in which a target of Tl-203 is irradiated in a cyclotron to produce Pb-201 through a (p,3n) reaction. Lead-Pb-201 is separated from the thallium target, and the purified Pb-201 decays to Tl-201. The process takes advantage of the ability of elemental thallium in the (I) and (III) oxidation states and the different chemical properties of each species.

Synthesis and purification of (DOTA)-Re(Arg11)CCMSH. The synthesis of this α-MSH analog has been published (Chen et al., 2001). A protein synthesizer is required. Such capabilities exist at two locations in St. Petersburg, including the Institute of High-Molecular-Weight Compounds, and St. Petersburg State University. The peptide's primary structure is stabilized through formation of a Re complex with several amino acid side groups. The DOTA is conjugated to the peptide using standard techniques for forming the covalent bond between the peptide and DOTA. The synthesized conjugate is then purified by HPLC techniques to yield a pure peptide.

Synthesis of Pb-203-labeled peptide. The procedure for coordinating lead to the peptide has been published (Miao et al., 2005). A solution of Pb-203 was substituted for Pb-212 to make the Pb-203-labeled peptide.

Characterization of Pb-203-labeled peptide. The synthesized Pb-203-labeled peptide was purified by HPLC. The Pb-203-labeled peptide and the unlabeled peptide have different retention times, and the specific activity of the labeled peptide is high. The purified labeled peptide was assayed to determine the labeling yield (amount of Pb-203 incorporated into the conjugated peptide versus the amount remaining unbound) using ITLC and HPLC. The radiolabeled peptide was also evaluated for toxicological parameters using standard drug product tests.

Data Analysis. Results from the production of Pb-203 synthesis in Phase I, including purification and characterization of the peptide and its conjugate, imaging studies, biodistribution measurements, and

dosimetry calculations were used to evaluate the potential application of Bi-203-labeled peptide as an imaging agent.

Biodistribution and dosimetry of Pb-203-labeled peptide. In follow-on work, the Khlopin Radium Institute will conduct studies on the biodistribution of Pb-203-peptide. The role of PNNL will be to review the biodistribution and pharmacokinetic data obtained by Russian scientists for Pb-203-labeled peptide, and to calculate retrospective radiation doses using anthropomorphic (generic) models recommended by the Medical Internal Radiation Dose Committee of the Society of Nuclear Medicine. Pacific Northwest National Laboratory will also evaluate the dosimetry of Pb-212/Bi-212-labeled peptide in patients administered peptide co-labeled with Pb-203, which serves as the imaging radioisotope.

Pb-212/Bi-212 generators. Pacific Northwest National Laboratory prepared and shipped radium-224 generators from which lead-212 and its daughter product bismuth-212 were obtained for this research. The generators were shipped to the Khlopin Radium Institute in St. Petersburg, Russia.

Synthesis of Pb-212-labeled peptide. The Khlopin Radium Institute prepared Pb-212-labeled peptide using the same procedure used to prepare Pb-203-labeled peptide and a more robust cation-exchange column capable of withstanding the anticipated alpha radiation dose to the column from Bi-212.

Characterization of Pb-212-labeled peptide. At Khlopin, the synthesized Pb-212-labeled peptide was purified by HPLC. The specific activity of the labeled peptide was high. The purified labeled peptide was characterized with respect to the labeling yield (amount of Pb-212 incorporated into the conjugated peptide versus the amount remaining unbound) using HPLC.

Biodistribution and dosimetry of Pb-212-labeled peptide. In follow-on studies, The Khlopin Radium Institute will perform biodistribution studies of Pb-212-labeled peptide in normal and immunodeficient mice inoculated with B16/F1 melanoma tumor cells to produce xenografts. Treated mice and a control group will be administered purified labeled peptide to determine the organ biodistributions, target-to-nontarget tissue ratios, and drug pharmacokinetics. Pacific Northwest National Laboratory will calculate radiation doses from these data and predict the behavior and dosimetry of the radiolabeled peptide in man.

Research Results and Findings

Lead-203 production. Using the MGC-20 cyclotron, lead-203 was produced by proton irradiation of thallium-203 by the reaction $^{203}Tl(p,n)^{203}Pb$ to yield about 10 MBq/μA-hr. The short-lived isomers ^{202}Pb (half-life = 3.53 hr) and ^{204}Pb (half-life = 67.2 min.) were identified. To improve target heat transfer, the thallium target was pressed against a platinum backing. Lead-203 was extracted from the target by dissolution in HCl followed by cation exchange on a resin ion-exchange column. The column binds lead, while thallium passes through. A second solution of HCl with acetone recovers lead-203 with traces of thallium, which is then passed through an anion-exchange column, which retains thallium and allows purified Pb-203 to pass through. Lead-203 may also be co-precipitated with iron hydroxide, followed by thallium removal by centrifugation. Lead-203 extraction from the carrier may then be made by anion exchange. Production and extraction by the co-precipitation methods was found to be more efficient and cost-effective.

Synthesis and purification of the DOTA-Re-CCMSH peptide. The peptide preparation, purification, and characterizations were performed with the assistance of the DIAPHARM Company in St. Petersburg.

The Khlopin Radium Institute acquired and used a high efficiency liquid chromatography system to characterize the quality and purity of the synthesized peptide.

Preparation of lead-203-labeled peptide. Common methods for peptide labeling with radioactive lead produce low yields. A new method was developed using a method without a limiting solvent-concentration gradient. The eluent acetonitrile (21-23%) in a 0.1% water solution with trifluoroacetic acid provided a good environment for lead-203 labeling and separation of unreacted contaminants. Measurements of labeled-peptide yield were made using gamma spectrometry. The method was improved by optimizing the acetate buffer pH and reaction solution temperature. The best results were obtained at pH = 6-6.2 at 70-80 °C for one hour. DOTA easily complexes with metal contaminants in the reaction mixture. Therefore, special effort must be made to minimize metal atoms prior to labeling with Pb-203. Special attention must be paid to the purity of all chemical reagents used. Excess lead must be removed from the enriched thallium target. With optimization, product yields of 90-95% were obtained on this study.

Toxicity of lead-203-labeled peptide. The radiolabeled peptide was evaluated for pH, presence of reducing impurities, changes in optical density, potentially toxic chemical compounds, and standard laboratory toxicological parameters, including acute toxicity, cytotoxicity, chronic toxicity, presence of irritants, sensitizing effects, hemolytic activities, pyrogenicity (fever induction), and sterility (bacterial contamination). All tests were negative for drug-product toxicity. The radiolabeled peptide was pyrogen-free and sterile.

Biodistribution of lead-203-labeled peptide in live animals (laboratory mice).

We evaluated the biodistribution of intraperitoneally administered ^{203}Pb-DOTA-Re-CCMSH- MSH(0.1 mL) in intact (live) animals (black female mice C57 CBAxBl) weighing 16-18 g. The biodistribution at various times post-injection are shown in Table 1. The drug accumulated quickly in the intestines, and at 24 hours was completely excreted through the bladder and intestines.

Table 1. Biodistribution of the drug MSH-Pb-203 in intact female mice C57 (CBAxB1) after intraperitoneal injection (percent of administered activity, corrected for radiological decay).

Organs and tissues	Time post-injection		
	1 hr	3 hrs	24 hrs
Blood	2.10	1.10	0.60
Thyroid	0.42	0.53	0.53
Liver	3.60	2.30	0.80
Kidney	1.00	0.50	0.10
Bladder	-	17.8	80.0
Stomach	1.40	1.60	7.00
Small intestine	44.20	17.60	-
Large intestine	4.80	23.10	-
Muscle	16.80	6.90	1.30
Skin	15.00	1.80	0.60
Washout			80.00

Transplantation of experimental tumors and biodistribution of radiolabeled peptide.

The biodistribution of lead-203-labeled peptide was evaluated in mice with tumor xenograft implants to determine whether the presence of tumor affected drug biodistribution. A 0.2 mL melanoma tumor cell suspension was injected subcutaneously into the lateral surface of female C57 mice under sterile conditions. Lead-203 peptide (0.1 mL) was administered intraperitoneally into the mice for 9-12 days after tumor implantation. Table 2 shows the biodistribution results. This study showed nearly identical behavior of radiolabeled petide in normal compared to tumor-bearing animals. This study also showed successful uptake and targeting of the anti-melanoma peptide in tumors.

Table 2. Biodistribution of the drug MSH-Pb-203 in the tumor-bearing mice after intraperitoneal injection (percent of administered activity, corrected for radiological decay).

Organs and tissues	Time post-injection						
	10 min	30 min	1 hr	3 hrs	5 hrs	24 hrs	48 hrs
Blood	3.21	3.15	2.80	2.45	2.18	0.13	0
Thyroid	0.23	0.69	0.69	0.5-3.2	1.9-3.7	-	-
Liver	13.53	9.40	7.99	10.57	5.43	0.41	0.23
Kidney	2.15	1.03	1.43	0.37	8.65	trace amount	0
Bladder	-	-	13.28	13.19	17.79	-	-
Stomach	1.41	3.81	1.74	1.82	2.19	0.05	0.02
Small intestine	11.54	25.05	27.48	60.74	30.07	0.22	0.13
Large intestine	1.25	0.97	0.88	5.44	10.76	0.28	0.20
Muscle concentration %/g	1.43-7.12	0.90-6.42	1.06-5.40	0.54-3.64	0.88-5.94	0.01-0.66	0
Skin concentration %/g	2.85-2.90	5.02-10.10	2.02-3.62	2.86-5.58	0.96-1.87	1.10-1.28	0
Tumor concentration %/g	1.57- 2.11	2.82-3.28	1.9-3.32	2.29-4.19	3.65-4.26	3.58-5.81	2.10-2.70
Washout						90.50	94.20

In melanoma cells, the radiolabeled peptide reached a maximum uptake and accumulation at about 2 hours post-injection, and was a factor of three greater than the accumulation in unpigmented cells (see also Wraight et al, 1992).

The biodistribution of the Pb-203-peptide in mice and in transplanted melanoma cells showed that after intraperitoneal administration, the labeled peptide was quickly excreted from normal organs and tissues, but accumulated predominantly in tumor cells. The concentration reached 2-3% administered activity/gram at 1 hour, and increased to 5% /gram at 24 hours. By 24 hours, most of the non-specific activity had cleared from normal organs and tissues. These are highly positive experimental results for a radiolabeled drug for cancer treatment.

Radium-224 generator testing. The radium-224 generator elutes lead-212 which decays to bismuth-212. The Khlopin Radium Institute received and tested the radium-224 generator. Shipment delays of three weeks for generator package from Richland to St. Petersburg may have caused excessive radiation damage to the column from alpha particles, which may have affected generator operation. The project intent was to use lead-212 for reactions with the peptide complex.

The radium-224 generator comprises a lead shield with ion-exchange resin inside containing the Ra-224 source (half-life = 87.8 hours). Tubing attached to the resin column allows the column to be washed with normal saline to elute lead-212. The generator column is first rinsed with 0.5 M hydrochloric acid to remove bismuth-212 and daughter products. Next, the lead-212 is eluted in 2 M hydrochloric acid. After the generator delivery to the Khlopin Radium Institute in February 2012, the first milking produced about 22 MBq of lead-212. However, further attempts to extract isotopes resulted in failure, as the generator column clogged and lost its permeability for elution. We presume that due to transportation delays and the length of customs clearance, generator ion-exchange resin was exposed to alpha-particle radiation and column degradation. The amount of lead-212 extracted from the generator allowed limited peptide labeling and valuable experience using and testing the generator system.

Synthesis and purification of Pb-212-labeled peptide.

We characterized the lead-212-peptide for labeling yield, purity, and other properties by chromatography using the same methods described above for lead-203-labeled peptide. Six peptide synthesis runs provided labeling efficiencies of 60-90%. Problems were encountered because the radioisotope is carrier-free. Losses of lead-212 occurred due to nonspecific sorption of radioisotope nanoquantities on the walls of the laboratory glass ware, and additional losses of lead-212 occurred during radioisotope transfer from one vessel to another. This problem was solved by adding a special ion-carrier (sodium nitrate), and losses of lead-212 before peptide labeling decreased to about 10%. Other challenges were encountered due to the high specific activity of lead-212, as well as features of the labeling reaction (small volumes of mixtures, high reagent concentrations, and alpha-particle radiolysis). Solution destruction was observed at all the stages of synthesis, especially after its purification and concentration in the HPLC column.

Biodistribution of lead-212-labeled peptide in tumor-bearing animals.

The biodistribution of lead-212-labeled peptide was assessed in C57 black female mice inoculated with melanoma. Results were similar to those observed using the lead-203 peptide level. These results were expected. Biodistribution was characterized by rapid clearance from blood and blood plasma (from 0.36 to 0.05 % administered activity per gram), fast clearance from normal organs and tissues, and a relatively high differential uptake and accumulation of the radiolabeled peptide in tumor cells (0.25 % per gram compared to 0.01 to 0.05% per gram in surrounding tissues. These results in laboratory animals show that future application of the radiolabeled peptide for treating metastatic melanoma appears promising.

Conclusions

The research performed on this study confirmed goals and objectives of the study. In summary, lead-212-labeled-[DOTA]-Re(arg^{11})CCMSH represents a novel class of metal-cyclized melanotropin peptide analogues with important application for imaging and treatment of melanoma. Alpha particles emissions from the first daughter bismuth-212 effectively irradiate targeted cells, indicating the importance of the radiolabeled peptide against metastatic disease.

Lead-203 was found to be an ideal radioisotope label for imaging and quantitative dosimetry of the administered therapeutic peptide against melanoma. Lead-203 may be produced by cyclotron irradiation of an enriched thallium-203 target using 16 MeV protons and a 10 μA current. With target cooling, we

achieved a lead-203 yield of about 100 MBq/hour. Two methods were developed and tested for lead-203 separation from thallium target material. The co-precipitation method showed advantages over ion exchange.

The peptide complex DOTA-Re-CCMSH was manufactured and tested for composition, homogeneity, purity, and reactivity. Labeling yields with lead-203 of 90% were achieved. The same techniques may be used for labeling the peptide with lead-212, although radiolysis can be problematic with the alpha-emitter (bismuth-212) daughter.

The production of lead-212 using the AlphaMed radium-224 generator system was demonstrated, although logistical problems associated with long-distance, international transportation must be overcome to reduce transit times and radiolysis of the ion-exchange column. An alternative to lead-212 production using the radium-224 generator is to produce lead-212 by electrostatic precipitation of lead-212 from radon-220, a daughter product of thorium-228 decay. On this project, substantial lead-212 was produced by this method for peptide labeling studies.

Lead-203- and lead-212-DOTA-Re-CCMSH were prepared, sterilized, and tested for preclinical studies in normal and tumor-bearing mice. This work provided important pre-clinical biodistribution data, confirming the rapid clearance of the radiolabeled peptide from blood and normal organs and tissues, and the accumulation of radiolabeled peptide in tumors.

This research also confirmed the relative safety of the radiolabeled peptide as a drug product in terms of various toxicological parameters (absence of acute toxicity, cytotoxicity, chronic toxicity, irritant, sensitizing effect, hemolytic activities), as well as the compound's apyrogenicity, and sterility. Based on these results, the radiolabeled peptide appears suitable for further studies, including clinical trials.

Technology Enhancements

The following technology enhancements were developed on this project:

1. Improved methods, reaction mixtures, compositions, and conditions of the procedure for preparing and radiolabeling the anti-melanoma peptide. Increasing the target product yield improves the efficiency of the peptide complex labeled with Pb-203 (for imaging) and Pb-212 (for therapy) production.

A. Increasing the target product yield for the reaction of the peptide complex DOTA-Re-CCMSH labeling with Pb-203/212.
B. Identifying improved reaction conditions: the labeling reaction was conducted in the acetate buffer at pH=6 to7 at 70 to 80^{o}C, using lead chloride.
C. Use of citrate buffer at pH=6 to 6.2, lead in the form of a nitro-acid salt, and increasing the reaction temperature 90^{o}C.

2. Using an auxiliary peptide in the procedure for preparing and labeling the peptide with lead-203/212. Increasing the target product yield improves the efficiency of the labeled peptide complex production.

A. Increasing the target product yield and its molar activity in the reaction of the peptide complex DOTA-Re-CCMSH labeling with Pb-203/212.
B. Labeling reaction is strongly influenced by presence of the side heavy metal trace contaminants
C. Using an auxiliary DOTA-group containing peptide for preliminary binding of contaminants in the reaction mixture. After the reaction with auxiliary peptide the DOTA-Re-CCMSH complex is added into the reaction mixture.

Combined application of these innovations increases the target product yield in labeling reaction from 10% to 90%, and improves the efficiency of the labeled peptide complex production by approximately an order of magnitude.

Partners Evaluation of Project (Dr. Herbert Moore, President and CEO, AlphaMed Inc.)

On the importance of this research and results obtained:

> "AlphaMed hopes to soon begin Phase I and Phase II clinical trials using the lead-212/bismuth-212-peptide against malignant melanoma. Aspects of the results from the Khlopin Radium Institute provide *independent* confirmation of earlier results from the University of Missouri. Independent confirmation from a separate organization, sans conflict of interest, is extremely important, and will be of considerable value in our presentations to the FDA."

On the scientific contributions of the Khlopin Radium Institute to the study:

> "In addition to confirming the University of Missouri results, mentioned above, KRI developed a technique for overcoming low reaction yields that intermittently plagued our efforts for years. Their innovation has been invaluable, and allowed us to realize consistently high yield reactions necessary for substantial and consistent tumor up-take."

On research future needs following completion of this study:

> "Being able to initiate clinical trials in Russia might have accelerated approvals in North America. The DOE's reluctance to be associated with human trials is understandable, but if that could be overcome the opportunity to conduct trials in a patient population unencumbered by previous North American treatment regimens would be of value."

Future Plans and Commercialization Activities

The alpha-particle-emitting peptide lead-203- and lead-212-DOTA-Re-CCMSH represents a potentially attractive therapeutic agent against malignant melanoma in humans. No other current drug or radionuclide approach provides equivalent or better promise for treating metastatic melanoma. AlphaMed would like to pursue an investigational new drug (IND) application to the U.S. Food and Drug Administration for clinical testing. The company will be developing commercialization plans for further development, and will be seeking financial partners to help finance this development and drug application process. AlphaMed would like to continue working with PNNL and the Khlopin Radium Institute (and its Russian collaborators) on this effort.

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Subject Invention and Participating NIS Institute Invention Summary

No Subject Inventions or Participating NIS Institute Inventions were derived under the project.