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TITLE: Targeted Gold Nanoparticles (AuNPs) for Potent Alpha-Particle Radiotherapy of Brain Cancer

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CONTRACTING ORGANIZATION: Duke University, Durham, NC

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14. ABSTRACT

Glioblastoma (GBM) is the most common and aggressive brain cancer. Even with the highest first-year cost (> $120,000) under standard-of-care treatments, the prognosis for GBM patients is dismal. Therefore, it is of great clinical significance to develop novel therapeutic approaches to improve GBM treatment efficacy. Alpha particle radiation therapy with high linear energy transfer (80 keV/μm) has a potent therapeutic effect independent of dose rate, cell cycle, and oxygen concentration. A single alpha-particle track can result in lethal DNA double-strand breaks. Astatine-211 (^{211}At) is an attractive alpha emitter for alpha particle radiation therapy because it has the advantages of an optimal half-life (7.2 h) and no long-lived decay "daughter" radionuclides thus avoiding toxicity from daughter radionuclide redistribution. However, traditional ^{211}At radiolabeling methods focusing on At-C chemical bonds have the challenges of having a complicated radiolabeling process and low conjugation efficiency. In this study, we develop targeted gold nanoparticles as a novel ^{211}At delivery nanoplatform for alpha particle radiation therapy. In the past year, we have demonstrated that the developed gold nanoparticles can selectively accumulate in the brain tumor but not surrounding healthy brain tissue. We also performed preliminary in vivo toxicity study and therapeutic efficacy test. Preliminary experiment results demonstrated that alpha emitter radiation therapy with ^{211}At-loaded gold nanoparticles can substantially reduce tumor growth after intratumoral administration using murine animal models.

15. SUBJECT TERMS
Brain Cancer, Nanoparticles, At-211 alpha particle radiation therapy.

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1. INTRODUCTION:

Glioblastoma (GBM) is one of the most common and aggressive brain cancer with more than 10,000 newly diagnosed patients in the United States each year. The median survival is only 15 months even after aggressive treatments including surgery, chemotherapy, and radiation therapy. There is a clear and urgent need to develop novel therapeutic approaches for effective GBM treatment. Alpha particle radiation therapy has the promise to improve brain cancer treatment with its potent cytotoxicity from high linear energy transfer. Among the different available alpha-particle emitters, astatine-211 (^{211}At) has the advantage of optimal half-life (7.2 h) and no confounding radioactive daughters. This project is aimed to develop targeted gold nanoparticles as a novel ^{211}At delivery platform to treat brain cancer using murine animal models.

2. KEYWORDS:

Brain Cancer, Glioblastoma (GBM), Alpha particle radiation therapy, Astatine-211 (^{211}At), Gold nanoparticles (AuNPs).

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major goal 1: Develop AuNPs for At-211 alpha particle radiation therapy.

Milestone 1.1: Obtain AuNPs with different sizes for radiolabeling. (Target date: Dec 14th, 2019; Completed on Dec 1st, 2019).

Milestone 1.2: Get optimized AuNPs with high radiolabeling efficiency. (Target date: May 14th, 2020; Completed on April 30th, 2020).

Major goal 2: Functionalize AuNPs for brain cancer targeting and treatment.

Milestone 2.1: Functionalize AuNPs with both c(RGDfK) and angiopep-2 ligands. (Target date: May 14th, 2020; Completed on May 1st, 2020)

Milestone 2.2: Characterize AuNP's properties (Target date: May 14th, 2020; 80% completed).

Milestone 2.3: Demonstrate developed AuNPs can target and treat brain cancer cells with in vitro test (Target date: August 14th, 2020; 70% completed).

Major goal 3: Evaluate brain cancer targeting, pharmacokinetics, and therapeutic effect of developed AuNPs using murine animal models.

Milestone 3.0: Get animal protocol approved by Duke IACUC and DOD ACURO. (Target date: Nov14th, 2019; Completed on Mar 2nd, 2020)

Milestone 3.1: Get in vivo biodistribution and pharmacokinetic properties of AuNPs. (Target date: Nov 14th, 2020; 50% completed)

Major goal 3.2: Obtain MTD of ^{211}At-AuNPs. (Targeted date: November 14th, 2020; 30% completed)

Major goal 3.3: Determine the therapeutic effect of the developed ^{211}At alpha-particle radiotherapy with AuNPs and prepare a manuscript for publication. (Targeted date: February 14th, 2020; 40% completed)

What was accomplished under these goals?

Aim 3: Evaluate *in vivo* brain cancer targeting, pharmacokinetics, and therapeutic effect of targeted AuNPs labeled with both ^{211}At and ^{124}I.

Major task 1: Investigate brain cancer uptake, biodistribution, and pharmacokinetics of targeted AuNPs by microPET/CT imaging and using the GBM brain tumor model with U87MG brain cancer cells.

We radiolabeled gold nanoparticles (gold nanostars) with ^{124}I and performed microPET/CT scan to investigate brain cancer uptake, biodistribution, and pharmacokinetics. The radiolabeling process was performed by mixing ^{124}I with gold nanoparticles. The radiolabeled gold nanoparticles were purified by centrifugation wash. For PET/CT scan, radiolabeled gold nanoparticles were injected through tail vein and mice with brain tumor were imaged 10 min, 4 h, 24 h, 48 h, and 120 h after IV injection. Gold nanoparticles were found in brain tumor part but not surrounding healthy brain tissue (Figure 1).

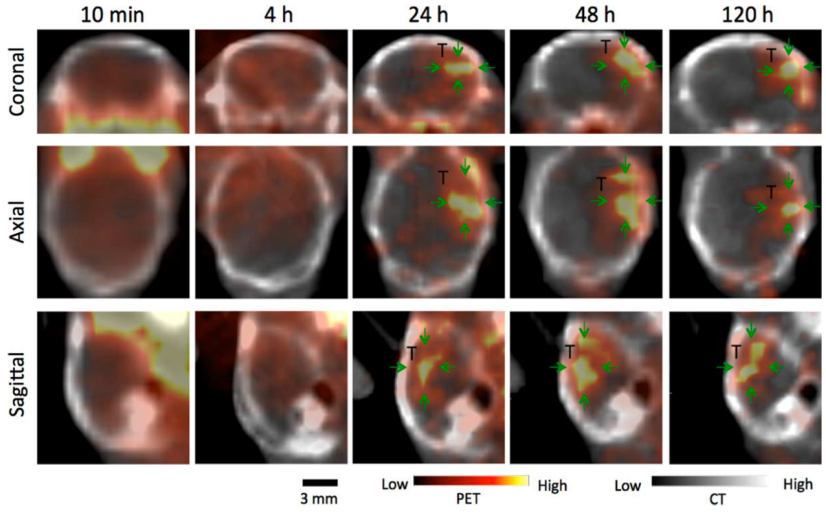

Figure 1. PET/CT imaging of radiolabeled gold nanoparticles in the implanted brain tumor 10 min, 4 h, 24 h, 48 h, and 120 h after intravenous injection. Top, middle and bottom rows show coronal, axial and sagittal image, respectively. Significantly higher ^{124}I-GNS uptake in tumor (T; green arrows) compared with contralateral normal brain was observed 24 h, 48 h and 120 h after IV injection.

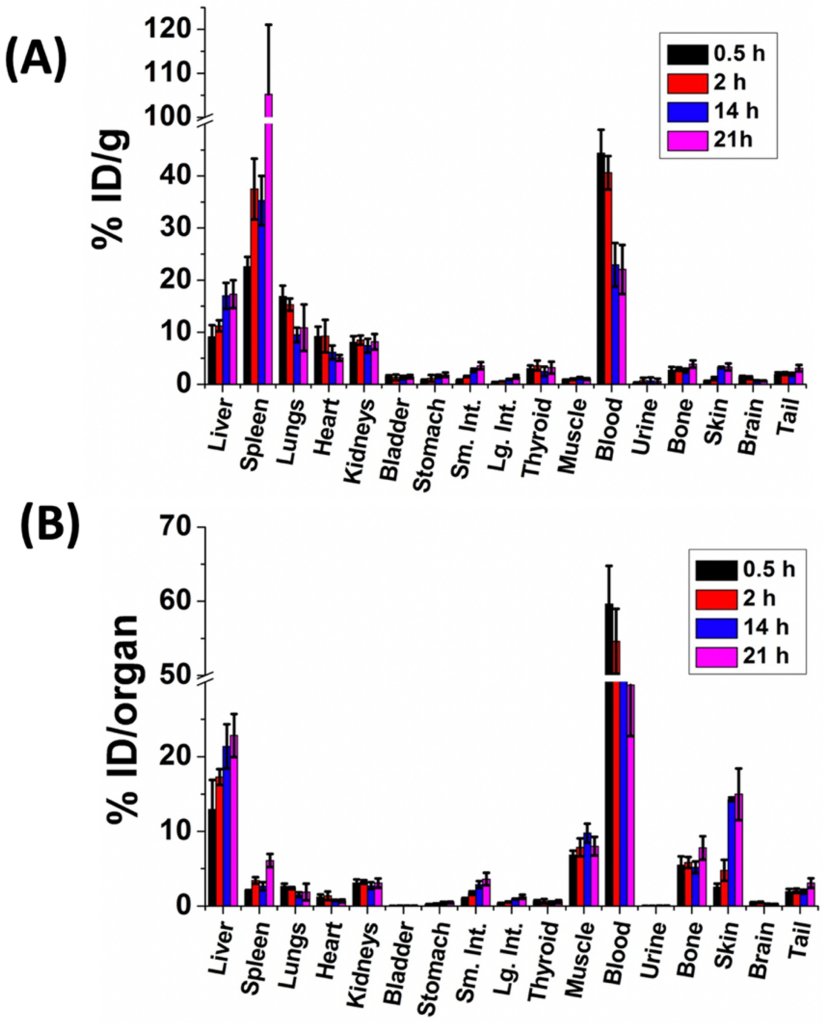

Figure 2. Biodistribution of ^{211}At activity after intravenous injection of ^{211}At-labeled gold nanoparticles in normal mice. The results are shown as percent injected dose per gram tissue (%ID/g) [Figure (A)], and as percent injected dose per organ (%ID/organ) [Figure (B)]. Error bar shows the standard deviation (n=5).

In addition, we also performed biodistribution study for ^{211}At radiolabeled gold nanoparticles after intravenous injection. 4 time pointes were studied (0.5 h, 2 h, 14 h, and 21 h). The radiolabeling efficiency of ^{211}At on gold nanostars was almost 100% using a simple and rapid synthesis process that was completed in only 1 min. *In vitro* stability test in serum showed that more than 99% of the ^{211}At activity remained on the gold nanostars after 24 h incubation at 37°C. As shown in Figure 2, *in vivo* biodistribution results showed low uptake in the thyroid (0.44-0.64 %ID) and stomach (0.21-0.49 %ID) between 0.5 h and 21 h after intravenous injection, thus indicating excellent *in vivo* stability of ^{211}At-labeled gold nanoparticles.

Major task 2: Determine the maximum tolerated dose (MTD) of ^{211}At-AuNPs.

We performed preliminary *in vivo* toxicity study to determine the MTD of ^{211}At radiolabeled gold nanoparticles after intravenous injection. 3 doses of ^{211}At (0.3 mCi/kg, 1 mCi/kg, and 4 mCi/kg) were tested. Gold nanoparticles without ^{211}At were used as control. As shown in the Figure 3, 3 of 5 mice died within 9 days after IV injection of 4 mCi/kg ^{211}At radiolabeled gold nanoparticles. For the 1mCi/kg group, 2 of 5 mice died within 9 days after IV injection. For the 0.3 mCi/kg group and control group, none of the mice died and the body weight has no obvious decrease.

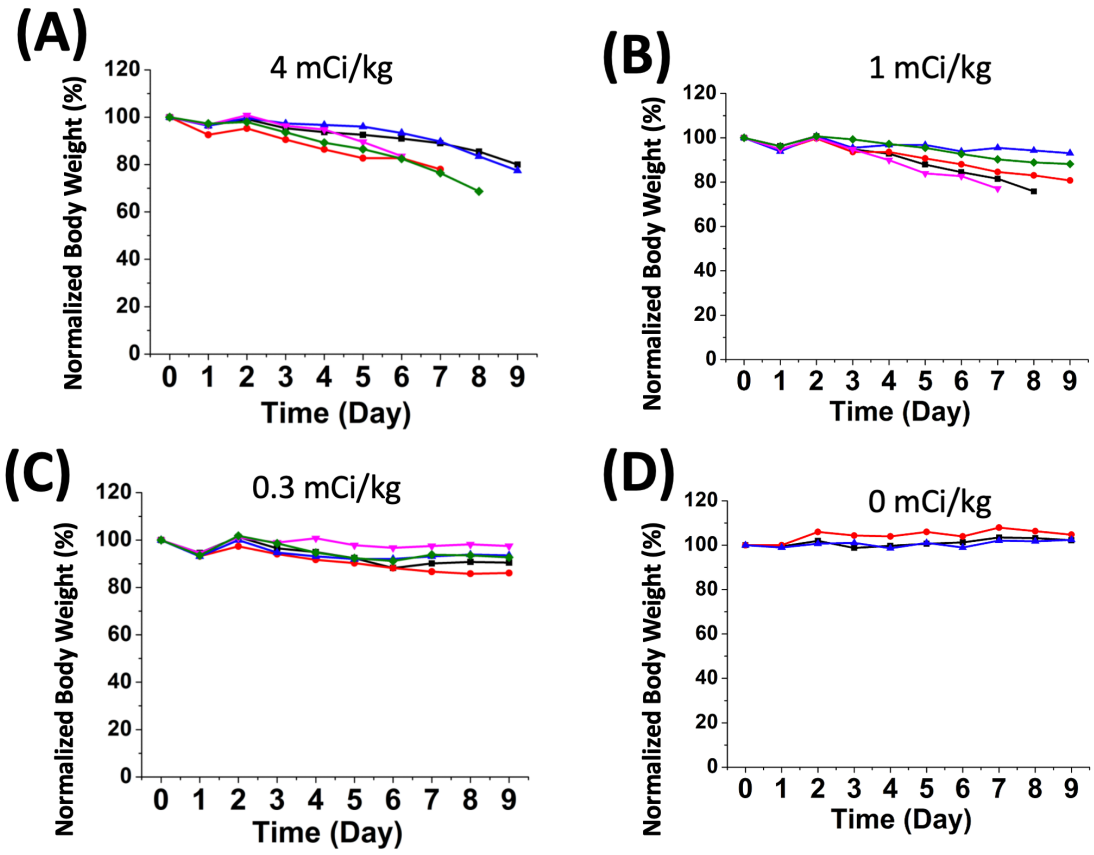

Figure 3. Body weight monitoring for mice with systemic administration of ^{211}At radiolabeled gold nanoparticles through tail vein. The ^{211}At dose ranging from 0.3 mCi/kg to 4 mCi/kg were tested. The body weight for mice with the 0.3 mCi/kg dose shows minimal body weight decrease.

Major task 3: Determine therapeutic response for targeted AuNPs with both ^{211}At and ^{124}I labeling using GBM murine animal model with nude mice and U87MG cell line.

We performed an initial evaluation of the therapeutic potential of ^{211}At-labeled gold nanoparticles. The experiment was performed in athymic mice with subcutaneous U87MG human glioma xenografts with the labeled drug given by the intratumoral route. As shown in the Figure 4, the ^{211}At-labeled gold nanoparticles substantially inhibited the tumor growth rate. The average tumor volume in the control group increased rapidly from 82 mm^3 on Day 1 to 177 mm^3, 409 mm^3, 798 mm^3, and 1456 mm^3 on Days 4, 7, 10, and 14, respectively. In contrast, the average tumor volume in the treatment group increased slowly, from 78 mm^3 on Day 1 to 95 mm^3, 112 mm^3, 152 mm^3, and 189 mm^3 on Days 4, 7, 10, and 14, respectively. The two-way ANOVA test indicated that the difference in tumor volumes between the ^{211}At-labeled GNS treated group and the control group with PBS injection was statistically significant (P<0.001).

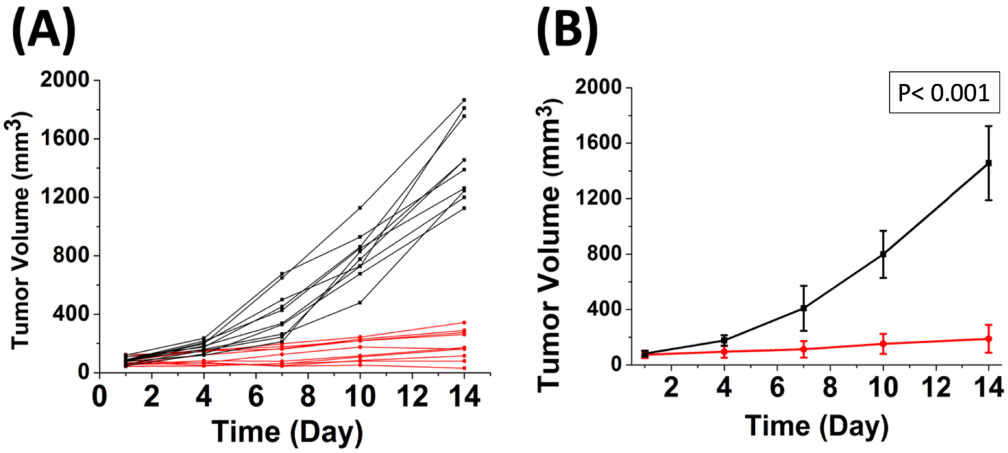

Figure 4. Therapeutic efficacy evaluation of ^{211}At TAT using gold nanoparticles as a novel delivery platform. (A) tumor size change profile for each mouse in the ^{211}At-GNS treatment group (30 μCi, red color line) and blank control group with PBS injection (black color line). (B) Average tumor size change profile for mice in the ^{211}At-GNS treatment group (30 μCi, red color line) and blank control group with PBS injection (Black color line). Error bar shows standard deviation (n = 10). P value was calculated using 2-way ANOVA (P< 0.001).

Summary

In summary, we have performed in vivo studies to investigate the brain cancer uptake, biodistribution, and therapeutic efficacy of gold nanoparticles loaded with ^{211}At. Preliminary results demonstrated that the ^{211}At radiolabeled gold nanoparticles could reduce tumor growth using a brain cancer murine animal model.

What opportunities for training and professional development has the project provided?

In the past year, the Horizon Award has provided me precious opportunities to take training for in vivo ^{211}At radiotherapy studies. I have learned how to perform in vivo pharmacokinetics, biodistribution, toxicity, and therapeutic efficacy studies. I have met my research mentors, Professor Tuan Vo-Dinh and Professor Michael Zalutsky, weekly to discuss project progress and received their professional comments and suggestions. I will attend the conference Pacifichem 2021 for the symposium entitled "Advancements in the Chemistry of Targeted Alpha Therapy" to learn the latest advancements in alpha particle radiation therapy using ^{211}At and other alpha emitters.

How were the results disseminated to communities of interest?

I have submitted a paper to the International Journal of Nanomedicine with the title of "Gold nanostars: A novel platform for developing ^{211}At-labeled agents for targeted alpha-particle therapy" to disseminate the results to communities of interest. The paper has been accepted.

In addition, I also submitted an abstract for the conference Pacifichem 2021 with the title of "Gold nanoparticles as a novel delivery strategy for targeted alpha therapy". The abstract has been accepted for an oral presentation to disseminate the results to communities of interest. I will attend the conference between December 16, 2021 and December 21, 2021.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next report period, I will finish in vitro tests including binding affinity and cytotoxicity. In addition, I will continue in vivo studies to finish this project by determining the maximum tolerable dose (MTD), investigating pharmacokinetics and biodistribution, and evaluating the therapeutic efficacy of ^{211}At-loaded AuNPs using murine animal models.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

From this project, we demonstrated that AuNPs can be used as a novel delivery platform for ^{211}At alpha particle radiation therapy. The developed strategy has the advantages of the simple radiolabeling process and high conjugation efficiency, which is attractive for targeted alpha particle therapy to treat cancer.

What was the impact on other disciplines?

We are first to explore gold nanoparticles as a novel ^{211}At carrier for in vivo cancer treatment. Preliminary results demonstrated that ^{211}At labeled gold nanoparticles can substantially reduce brain cancer growth. The optimized gold nanoparticles with high ^{211}At binding affinity may serve as a novel delivery strategy for alpha particle radiation therapy to treat aggressive cancer.

What was the impact on technology transfer?

The delivery nanoplatform developed in this project has the promise to make an impact by clinical translation to perform alpha particle radiation therapy with ^{211}At.

What was the impact on society beyond science and technology?

Results from this project could make an impact on society by improving public knowledge about cancer therapy. The targeted alpha particle radiation therapy is an emerging technology to improve cancer treatment efficacy.

5. CHANGES/PROBLEMS:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Due to the COVID-19 pandemic, the research progress was delayed. We plan to speed up the research activities to finish this project in the rest time.

Changes that had a significant impact on expenditures

The expenditures were less than expected because the research progress was delayed by the COVID-19 pandemic.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Human subjects are not involved in this project.

Significant changes in use or care of vertebrate animals

There are no significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

There are no significant changes in use of biohazards or select agents.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

We have one paper accepted for publication in the International Journal of Nanomedicine. Yang Liu, Zhengyuan Zhou, Yutian Feng, Xiao-Guang Zhao, Ganesan Vaidyanathan, Michael R. Zalutsky, Tuan Vo-Dinh. Gold nanostars: A novel platform for developing ^{211}At-labeled agents for targeted alpha-particle therapy. Accepted. The federal support is acknowledged in this journal publication.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

From this project, we developed gold nanoparticles as a novel delivery strategy for targeted alpha particle therapy to treat cancer. The developed technology was shared by publishing a peer-reviewed paper in the International Journal of Nanomedicine. In addition, I will share the developed technology by giving an oral presentation in the conference Pacifichem 2021.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Yang Liu
Project Role: Principal Investigator
Researcher Identifier (ORCID ID): 0000-0003-3640-8852
Nearest person month worked: 12
Contribution to Project: Yang Liu is the PI for this project and has been actively involved in the whole project including targeted nanoparticles development, radiolabeling test, in vitro, and in vivo studies.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Generic Award Chart:

W81XWH1910684: Targeted Gold Nanoparticles (AuNPs) for Potent Alpha-Particle Radiotherapy of Brain Cancer

PI: Yang Liu, Duke University, NC **Budget:** $240,041.00

Topic Area: FY 2018 DoD PRCRP, Horizon Award **Mechanism:** W81XWH-18-PRCRP-HA

Research Area(s): 0805, 0808, 0817 **Award Status:** 15-Aug-2019 To 14-Feb-2022

Study Goals:

The overall objective of this research is to develop a novel image-guided ^{211}At radiotherapy with targeted AuNPs (<5 nm) for brain cancer treatment.

Specific Aims:

1. Synthesize, optimize and evaluate ultrasmall AuNPs (< 5 nm) for ^{211}At and ^{124}I labeling.
2. Conjugate AuNPs with tumor-homing peptides for brain cancer targeting and perform in vitro tests to demonstrate the developed nanoagent can target and treat brain cancer.
3. Evaluate in vivo brain cancer uptake, biodistribution, pharmacokinetics, and therapeutic effect of targeted AuNPs with both ^{211}At and ^{124}I labeling using a murine animal model.

Key Accomplishments and Outcomes:

Publications: 1 peer-reviewed publication (Accepted)
Patents: none to date
Funding Obtained: none to date

9. APPENDICES:

Not applicable.