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TITLE: Detection and Treatment of Advanced Prostate Cancer with Radiolabeled Transferrin Molecules

PRINCIPAL INVESTIGATOR: Dr. Ning Zhao

CONTRACTING ORGANIZATION: The Regents of the University of California, San Francisco

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)

Dr. Ning Zhao
Michael Evans, PhD

E-Mail:Michael.Evans@ucsf.edu

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University
of California, San Francisco
1855 Folsom St. Ste 425
San Francisco, CA 94143-4249

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT We propose a new therapeutic paradigm for the treatment of castration resistant prostate cancer: targeting a shared pathobiological event upregulated by hyperactive MYC and mTOR with radiotherapy to ablate tumor cells. Because transferrin uptake into a cell is upregulated by hyperactive MYC or mTOR, we propose to synthesize a theranostic transferrin nanoparticle conjugate. A second objective is to show that we can image prostate cancer tumors with SPECT using the transferrin construct. The final objective is to show that the transferrin construct can ablate prostate cancer tumors with hyperactive MYC and/or mTOR.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	18	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The goal of this proposal is to develop a new therapy to treat castration resistant prostate cancer, the most advanced and lethal form of the disease. Our new therapy is a radiolabeled version of a naturally occurring serum protein called transferrin. We decided to develop a transferrin-drug conjugate foremost because two common drivers of prostate cancer (MYC and mTOR) strongly upregulate transferrin uptake in a prostate cancer cell. Therefore, we hypothesize that virtually all castration resistant prostate cancer patients will harbor disease with high avidity for transferrin. Moreover, MYC cannot be directly drugged, and mTOR inhibition slows tumor growth, but does not kill tumor cells. Consequently, there is an urgent need to develop therapeutic strategies targeting these prostate cancer drivers with agents capable of inducing tumor cell death. While using transferrin to bring a therapeutic radioisotope to prostate cancer is highly innovative, we anticipate a high likelihood of success. We are optimistic given that two radioisotopes (Xofigo®, Samarium lexidronam) that target remodeling bone were recently approved in the USA for the treatment of castration resistant prostate cancer, showing that a tumor response can be affected with radiotherapy in spite of potential damage to normal tissue. Our transferrin drug conjugate has the potential to be even more impactful, as it will address soft tissue and bony metastases, and our new human imaging data show clear resolution of prostate cancer lesions, with very little transferrin uptake in normal tissues (the historical drawback cited by the community against transferrin-drug conjugates).

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Theranostics, nuclear medicine, radioligand therapy, MYC, small cell neuroendocrine prostate cancer, iron dyshomeostasis

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Training-Specific Tasks: *(only applicable to training award mechanisms)*

Major Task 1: Training and educational development in prostate cancer research *(only applicable to training award mechanisms)*	**Months**	**Responsible person(s)**
Subtask 1: Present research at weekly group meetings with the Evans, VanBrocklin, and Wilson labs (50% complete)	1-24	Dr. Zhao

Subtask 2: Present research at the monthly group meetings among the "Body Research Interest Group" in the Radiology Department (50% complete)	1-24	Dr. Zhao
Subtask 3: Present research at monthly group meetings with the Ruggero lab. (50% complete)	1-24	Dr. Zhao
Subtask 4: Attend one national scientific meeting per year in relevant scientific field. (50% complete)	1-24	Dr. Zhao
Subtask 5: Audit "Cancer Imaging" course for masters students in the Radiology department at UCSF (50% complete)	12-24	Dr. Zhao
Milestone(s) Achieved: Presentation of project data at a national meeting	24	

Research-Specific Tasks:

Specific Aim 1: Synthesis and characterization of ^{177}Lu-transferrin-AGuIX conjugate.		
Major Task 1: Synthesis of ^{177}Lu-Tf-AGuIX and ^{177}Lu-albumin-AGuIX		
Subtask 1: Coupling of ^{177}Lu to Tf-AGuIX or Alb-AGuIX 50% complete	1-2	Dr. Zhao
Subtask 2: Purification of ^{177}Lu-Tf-AGuIX and ^{177}Lu-Alb-AGuIX 50% complete	2-3	Dr. Zhao
Subtask 3: Analysis of binding affinity of the radiolabeled constructs for the transferrin receptor in HepG2 cells Cell lines used: HepG2 (ATCC) Complete	3-5	Dr. Zhao
Major Task 2: In vivo characterization of micro and macrodoses of ^{177}Lu-Tf-AGuIX and ^{177}Lu-albumin-AGuIX		
Subtask 4: Obtain ACURO approvals Complete	1-5	Dr. Zhao
Subtask 5: SPECT/CT imaging and biodistribution studies of mice bearing DU145 and DU145 PTEN RNAi tumors with ^{177}Lu-Tf-AGuIX and ^{177}Lu-Alb-AGuIX Cell lines used: DU145 (ATCC) 5% complete	5-10	Dr. Zhao
Subtask 6: SPECT/CT imaging and biodistribution studies of Pb-Cre PTEN$^{lox/lox}$ mice with ^{177}Lu-Tf-AGuIX and ^{177}Lu-Alb-	8-12	Dr. Zhao

AGuIX		
Subtask 7: SPECT/CT imaging and biodistribution studies of mice bearing PC3 tumors with ^{177}Lu-Tf-AGuIX and. Mice will be treated prior to radiotracer injection with either vehicle or RAD001. Cell lines used: PC3 (ATCC)	12-14	Dr. Zhao
Subtask 8: SPECT/CT imaging and biodistribution studies of Pb-Cre PTEN$^{lox/lox}$ with ^{177}Lu-Tf-AGuIX. Mice will be treated prior to radiotracer injection with either vehicle or RAD001.	14-18	Dr. Zhao
Subtask 6: Determination of the maximum tolerated dose for 177Lu-Tf-AGuIX in normal mice	18-20	Dr. Zhao
Milestone(s) Achieved: feasibility studies showing the dependence of 177Lu-Tf uptake on mTORC1 and MYC activity in prostate cancer models. Determination of the dose for the studies in specific aim 2.	20	
Specific Aim 2: Proof of concept in vivo studies in animals bearing human prostate cancer models.		
Major Task 3: Therapy studies with ^{177}Lu-Tf-AGuIX and ^{177}Lu-albumin-AGuIX		
Subtask 1: Treat mice bearing PC3 tumors with ^{177}Lu-Tf-AGuIX and ^{177}Lu-albumin-AGuIX at the MTD and 0.5MTD.	18-22	Dr. Zhao
Subtask 2: Molecular analysis of tumor tissue to probe for markers of DNA damage and cell death.	22-24	Dr. Zhao
Subtask 3: Molecular analysis of tumor microenvironment to probe for markers of DNA damage and cell death.	22-24	Dr. Zhao
Milestone(s) Achieved: Proof of concept establishing that prostate cancer can be treated with Tf-targeted radiotherapy	24	

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. Major activities

As indicated in the statement of work, radiolabeled transferrin adducts have been synthesized and the initial functional characterization has begun. The functional characterization to date has included characterizing the outcome of the chemical synthesis, in vitro serum stability and binding studies. During the second reporting period, animal imaging and antitumor assessment studies will be performed and compared to analogous data acquired with the radiolabeled albumin negative control.

2. Specific objectives

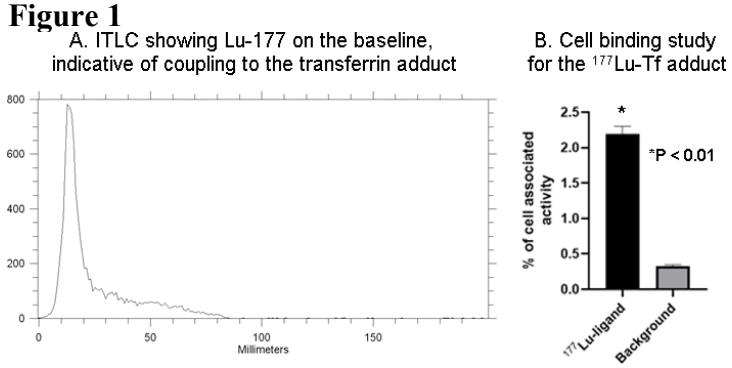
The specific goals of this project period were to complete the synthesis and in vitro characterization of the Lu-177 transferrin adducts. In addition, small animal SPECT/CT studies were to be performed to estimate radiotracer dosimetry and safety. Lastly, several training exercises were planned, including internal and external presentations of research progress.

3. Significant results and key outcomes

The synthesis of the Tf-AGuIX construct was performed using EDC to convert a carboxyl group on DOTAGA to form an O-acylisourea intermediate (87% yield). NHS was added to generate an amine-reactive NHS ester (quantitative yield). The amine-reactive NHS ester was reacted with a primary amine on a lysine within commercial holo transferrin, resulting in a stable amide bond. After purification with SEC, the conjugation of transferrin to AGuIX was confirmed by ESI mass spectrometry. An accurate quantification of Tf per particle was performed based on the absorption titration of Fe(III) chelated by the Tf, after separation by HPLC of the free Tf and free Fe(III) from the Tf-AGuIX, and shown to be ~5:1 AGuIX:Tf. Tf-AGuIX was incubated with $^{177}LuCl_3$ to append ^{177}Lu to the free DOTAGA chelators, and the reaction proceeded to >97% yield with >99% purity (**Figure 1A**).

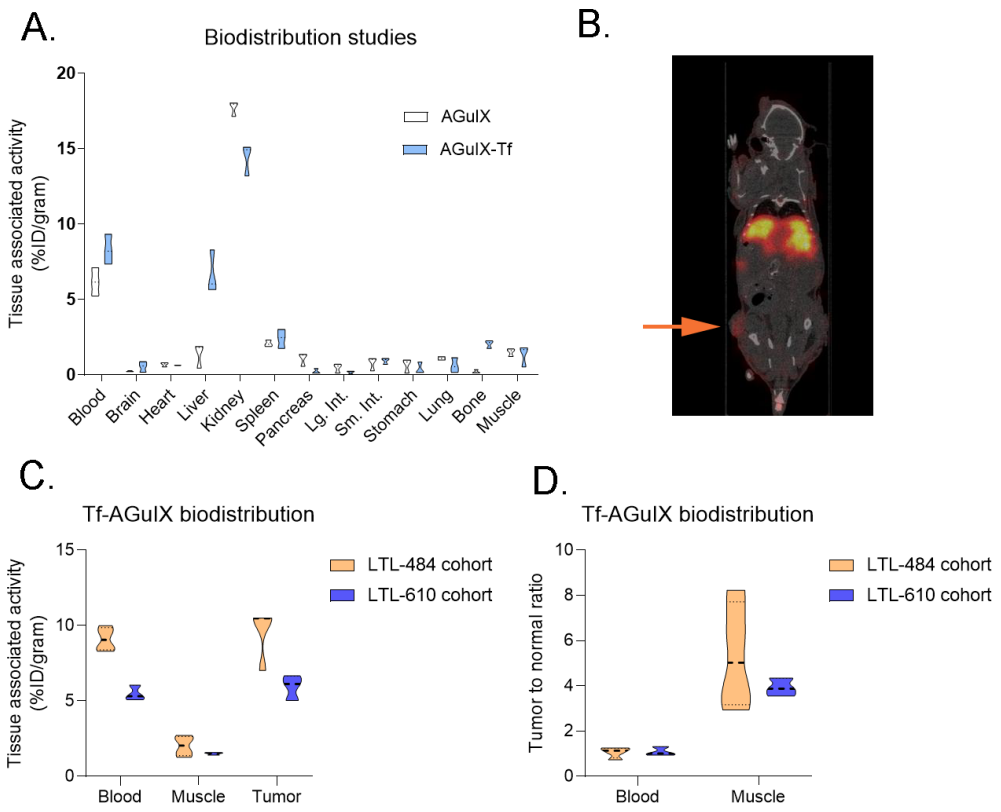
The affinity of ^{177}Lu-Tf-AGuIX for TFRC was determined relative to low specific activity ^{125}I-labeled Tf (prepared by direct iodination of tyrosine residues) using a cell-based assay. Briefly, HepG2 cells were incubated with ^{177}Lu-Tf-AGuIX or ^{125}I-Tf and competition for TFRC binding will be determined by adding a gradient of cold Tf to separate incubations. We found no significant loss in affinity of Tf for the transferrin receptor due to the functionalization with Lu-177 and AGuIX. Representative cell binding data are shown in **Figure 1B**, and we are currently working to fit the data to enable rigorous comparisons of the affinity of the Tf adducts.

Figure 1



The biodistribution of AGuIX and Tf-AGuIX were assessed in nontumor bearing and tumor bearing mice using SPECT/CT and post mortem tissue analysis. The uptake of either tracer was compared at 48 hours post injection (**Figure 2A**). The biodistribution was generally similar, with a few exceptions. For example, the liver uptake of Tf-AGuIX was significantly higher than what was observed for naked AGuIX. This is an expected finding as Tf and Tf-conjugates are known to traffic to the liver. The uptake of Tf-AGuIX in the kidneys of mice was significantly lower than AGuIX, which likely reflects the redistribution of the tracer to the liver owing to the binding properties of Tf. Tf-AGuIX uptake in the bone was also significantly higher than AGuIX, which may reflect binding to blood marrow cells that are not mechanically separated post mortem during the biodistribution studies. Tumoral uptake of Tf-AGuIX in a mouse bearing a PC3 tumor was visually obvious on SPECT/CT in a pilot imaging study (**Figure 2B,** tumor indicated with orange arrow). The data were acquired at 48 hours post injection. The biodistribution of Tf-AGuIX was assessed in two cohorts of mice bearing 484 or 610 tumor models. The uptake at 48 hours post injection was equal or higher than two normal reference tissue compartments, blood and skeletal muscle (**Figures 2C** and **2D**). Collectively, these data support the feasibility of pursuing antitumor assessment studies with Tf-AGuIX constructs.

Figure 2



What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Dr. Zhao has utilized this award to significantly expand his understanding of radiochemistry, radioligand therapy, and prostate cancer research. He has regularly attended and presented at Evans or Ruggero lab meetings, and he presents his research once every 2-3 months to the molecular imaging research community at UCSF. This is a "super group meeting" involving the Evans, VanBrocklin, Flavell, and Wilson labs at UCSF. Dr. Zhao has also presented his research at the UCSF Department of Radiology and Biomedical Imaging annual research symposium. Dr. Zhao was also invited to present some of his ongoing research as a virtual poster at the Society of Nuclear Medicine-India national meeting in December 2020.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

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

As COVID 19 restrictions are lessened, the objective of the next reporting period will be to conduct antitumor assessment studies.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The goal of this proposal is to develop a new radioligand therapy to treat castration resistant prostate cancer, the most advanced and lethal form of the disease. Our approach targets iron dysregulation, which is a highly common event in CRPC that occurs in both adenocarcinoma and neuroendocrine prostate cancer. We also understand that transferrin uptake occurs in CRPC that would not be expected to respond well to other radioligand therapies in clinical use, including bone seeking radionuclides and PSMA-directed radioligand therapy. Thus, we expect that our new therapeutic approach could bring a very effect treatment modality (i.e. radioligand therapy) to a broader swath of CRPC patients. Moreover, it is possible that CRPC that has relapsed on bone seeking or PSMA-targeted radioligand therapy may be responsive to transferrin receptor targeted therapy. In summary, we present a novel approach to radioligand therapy that may overcome the shortcoming associated with current treatments.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

There were obvious delays in performing some of the science due to COVID 19 restrictions. In addition to the delays due to limited onsite capacity in the laboratory, animal experiments were significantly delayed due to issues with ordering mice, radioactivity, or both. As COVID 19 restrictions ease, we have been able to resume a more vigorous working schedule with fewer delays in shipping. Although not a significant change, some additional tumor models were studied. These are human prostate cancer tumor models from commercial sources and de-identified.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

See the above comments regarding COVID 19 and the easing of restrictions at UCSF.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

COVID 19 restrictions delayed the purchasing of animals and radioisotopes for this study.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

-

Nothing to report

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Ning Zhao
- 8 calendar months

Dr. Zhao has performed the radiochemistry and characterization of new radiopharmaceuticals.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*