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TITLE: Precision Targeting of Castration-Resistant Prostate Cancer with a Novel Ferrous Iron-Dependent Therapeutic Delivery and Tumor Imaging Strategy

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14. ABSTRACT: We propose that mechanistically unrelated anti-cancer therapeutics can be more effectively deployed by administration in a pro-drug form that conditionally releases the therapeutic after chemical reaction with Fe(II), pools of which are augmented in CRPC cells and in the tumor microenvironment. To test and validate our hypothesis we will synthesize and evaluate in multiple prostate cancer models three novel agents, the Fe(II)-activated form of a potent DNA-alkylator (TRX-CBI), the Fe(II)-activated form of enzalutamide (TRX-ENZ), and a novel Fe(II)-targeted therapeutic radionuclide, 117Lu-TRX. We will also image prostate cancer in diverse animal models using an Fe(II)-activated PET probe (18F-TRX). Our objective is to show that castration resistant prostate cancer can be addressed effectively with these novel Fe(II)-targeted approach and that response to therapy can be predicted with 18F-TRX. Successful realization of these objectives via the IDA mechanism will greatly enable our long-term goal of identifying a "theranostic" development candidate that can be progressed toward first-in-human studies.

15. SUBJECT TERMS
NONE LISTED

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TABLE OF CONTENTS

1. INTRODUCTION:

We propose that mechanistically unrelated anti-cancer therapeutics can be more effectively deployed by administration in a pro-drug form that conditionally releases the therapeutic after chemical reaction with Fe(II), pools of which are augmented in CRPC cells and in the tumor microenvironment. To test and validate our hypothesis we will synthesize and evaluate in multiple prostate cancer models three novel agents, the Fe(II)-activated form of a potent DNA-alkylator (TRX-CBI), the Fe(II)-activated form of enzalutamide (TRX-ENZ), and a novel Fe(II)-targeted therapeutic radionuclide, ^{117}Lu-TRX. We will also image prostate cancer in diverse animal models using an Fe(II)-activated PET probe (^{18}F-TRX). Our objective is to show that castration resistant prostate cancer can be addressed effectively with these novel Fe(II)-targeted approach and that response to therapy can be predicted with ^{18}F-TRX. Successful realization of these objectives via the IDA mechanism will greatly enable our long-term goal of identifying a "theranostic" development candidate that can be progressed toward first-in-human studies.

2. KEYWORDS:

Molecular imaging, cancer theranostics, pro-drug, iron metabolism, fluorine-18, lutetium-177, pharmacology, castration resistant prostate cancer

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goal for project period one is listed below with the subtasks stipulated in the approved statement of work. An additional column, termed "status" has been added to indicate whether, or to what extent, the subtask has been completed. Where appropriate, specific dates have been added to indicate when the subtask was completed.

Specific Aim 1(specified in proposal)	Timeline	Site 1	Site 2	Site 3	Status
Major Task 1: In vivo evaluation of ^{18}F-TRX	Months				
Subtask 1: Synthesis of ^{18}F-TRX and ^{18}F-DXL	1-2	Dr. Renslo	Dr. Evans		Completed, Dec. 2018
Subtask 2: Breeding the colony of genetically engineered mice for imaging with ^{18}F-TRX and treatment with TRX-CBI	1-14			Dr. Ruggero (50 mice)	Ongoing
Subtask 3: PET and biodistribution studies in nu/nu mice bearing PC3 tumors with ^{18}F-TRX and ^{18}F-DXL Cell lines: PC3 (ATCC)	3-4		Dr. Evans (40 mice)		Completed, Feb. 2019
Subtask 4: PET and biodistribution studies of ^{18}F-TRX in mice bearing	4-6		Dr. Evans		Ongoing, 50%

xenografts: LNCaP-AR, PC3 and tumor sublines with stable MYC knockdown Cell lines: LNCaP-AR (Sawyers, MSKCC)			(20 mice)		completed
Subtask 5: PET and biodistribution studies of ^{18}F-TRX in mice bearing xenografts: DU145, LAPC4, and tumor sublines with stable PTEN knockdown Cell lines: DU145 (ATCC), LAPC4 (UCLA)	6-10		Dr. Evans (20 mice)		Ongoing, 50% completed
Subtask 6: PET and biodistribution studies of ^{18}F-TRX in genetically engineered mouse models	10-14		Dr. Evans	Dr. Ruggero (18 mice)	Completed,
Milestone(s) Achieved: Determination of tumor uptake of ^{18}F-TRX in vivo	14	Dr. Renslo	Dr. Evans	Dr. Ruggero	
Local IRB/IACUC Approval	2		Dr. Evans	Dr. Ruggero	Completed, Dec. 2018
Milestone Achieved: ACURO Approval	2		Dr. Evans	Dr. Ruggero	Completed, Dec. 2018

What was accomplished under these goals?

^{18}F-TRX detects prostate cancer tumor xenografts. We next evaluated if ^{18}F-TRX can detect human tumors derived from cell lines previously shown to harbor sensitivity to trioxolane pro-drugs. Biodistribution studies were first conducted in intact male nu/nu mice bearing subcutaneous PC3 xenografts at 30, 60, and 90 min post injection of ^{18}F-TRX. The uptake of the radiotracer steadily increased from 30 – 90 min post injection. Radiotracer levels in the tumor significantly exceeded blood and muscle at 90 min post injection with a tumor to blood ratio of 1.97±0.4 and a tumor to muscle ratio of 1.90±0.3 (**Figures 5A**).

We further tested if ^{18}F-TRX can detect PC3 implanted in the renal capsule, as subcutaneous implants, while convenient, do not model a clinically relevant tumor microenvironment. Biodistribution studies conducted 90 min post injection of ^{18}F-TRX showed equivalent radiotracer uptake in a PC3 tumor embedded in the renal capsule compared to the extent of uptake in subcutaneous PC3 tumors (**Figure 5B**). ^{18}F-TRX uptake was also significantly higher than background (blood and muscle) in subcutaneous EKVX and U251 tumors, two models of human lung adenocarcinoma and glioblastoma, respectively (**Figure 5B**). The U251 tumor, the model with the highest ^{18}F-TRX uptake, was visually obvious on small animal PET/CT (**Figure 5C**). Ex vivo analysis of the spatial distribution of the radiotracer in U251 tumors showed that ^{18}F-TRX was well distributed through the tumor xenograft, with the regions of highest uptake appearing to have the densest cellularity on H&E (**Figure 5D**). Collectively, these data show that genetically and pathologically diverse models of human cancer harbor high avidity for ^{18}F-TRX in vivo.

We next conducted an imaging study in a genetically engineered mouse model of prostate cancer. A 10 month old Pb-Cre:Pten$^{fl/fl}$ mouse with invasive adenocarcinoma was treated with ^{18}F-TRX, and after 90 minutes, the whole prostate was resected post mortem and imaged with PET/CT.

Radiotracer uptake was visually higher in the prostate tissue compared to seminal vesicles and muscle (**Figure 5E**). Moreover, ^{18}F-TRX uptake was predominant in the prostate tissue, and not observed in the cysts that routinely develop in this disease model.

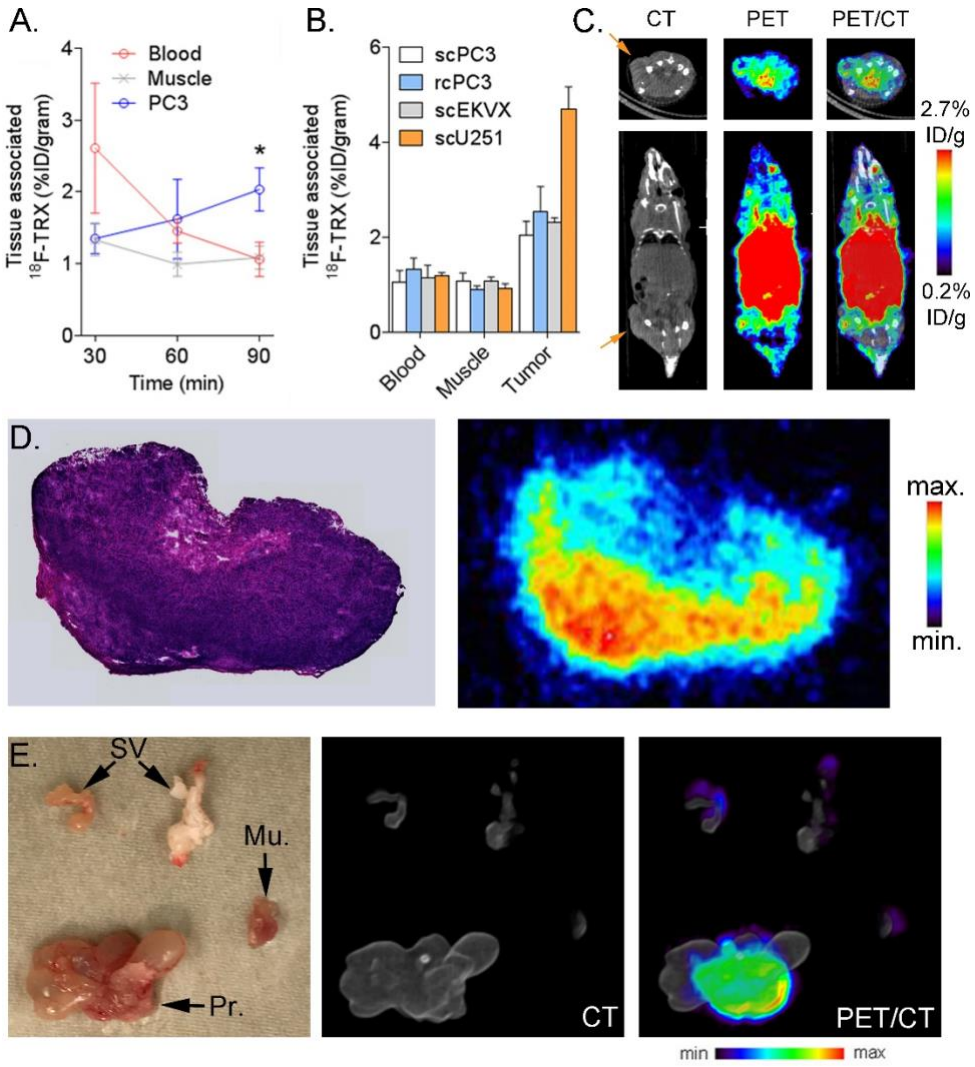

Figure 5. ^{18}F-TRX targets tumor tissue in vivo in genetically and pathologically diverse cancer models. Panel A. Biodistribution data acquired at 30, 60, and 90 min post injection of ^{18}F-TRX in male nu/nu mice with subcutaneous PC3 xenografts. The radiotracer uptake in the tumor continually increases from 30-90 min, consistent with a biochemical mechanism of action. Moreover, radiotracer uptake in the tumor exceeds the level observed in blood and muscle at 90 min post injection, two standard reference compartments for background radiotracer accumulation. The human prostate cancer model PC3 was prioritized as it was previously shown to be highly sensitive to an Fe(II)-sensitive TRX prodrug bearing a chemotherapeutic payload.[32] *P<0.01 compared to blood and muscle. **Figure S4** shows the biodistribution values for the entire repertoire of tissues from this animal cohort as well as the tumor to normal tissue ratios. Panel B. Biodistribution data acquired 90 min post injection of ^{18}F-TRX shows radiotracer uptake in tumor exceeding background for PC3 tumors implanted in the renal capsule (rcPC3), and subcutaneous EKVX and U251 tumors (scEKVX, scU251). **Figure S5** shows a MR image highlighting the tumor burden in renal capsule. Figure S6 shows the complete biodistribution data sets, and the tumor to normal tissue ratios. Panel C. PET/CT imaging data showing uptake of ^{18}F-TRX in tumor and normal tissues for mice bearing subcutaneous U251 tumors. The data was acquired at 90 min post injection. Panel D. H&E (left) and digital autoradiography (right) showing ^{18}F-TRX distribution within a representative section of U251 tumors. ^{18}F-TRX appears to be present in all regions of the slice, with the highest relative uptake appearing to co-localize with the area of densest cellularity on H&E. Panel E. (left) A photograph of the surgically excised whole prostate (Pr.), a piece of muscle from the hindlimb (Mu.), and the seminal vesicles (SV) of a ten month old Pb-Cre:Pten$^{fl/fl}$ mouse with fully invasive adenocarcinoma. Cysts extending from the anterior prostate are evident by eye. (middle) A volume rendered CT image of the tissues acquired on a small animal PET/CT. (right) A volume rendered PET/CT image of ^{18}F-TRX uptake in the tissues acquired 90 min post injection of ^{18}F-TRX. The image clearly shows relatively higher accumulation of radiotracer in the diseased prostate compared to muscle or seminal vesicles. ^{18}F-TRX was excluded from the cysts, as expected.

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

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

We will continue to establish the mouse prostate cancer models and conduct imaging and biodistribution experiments with ^{18}F-TRX. We will aim to report these studies in a follow-up manuscript to the first publication, which was primarily meant to establish proof-of-concept that Fe(II) can be imaged with PET in normal and a few select cancer models.

We will also begin testing the antitumor efficacy of TRX-CBI in prostate cancer animal models. To this end, the Renslo laboratory has begun synthesis of the therapeutic, and the Ruggero

lab has expanded a transgenic animal colony of mice with spontaneous prostate cancer. We also have all of the cell line models required to establish the animal models.

Lastly, we have begun the synthesis of a TRX conjugate for radiolabeling with Lu-177. We anticipate beginning pilot labeling studies during this project period, and preliminary mouse biodistribution studies prior to antitumor assessment studies.

4. IMPACT:

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

We have shown for the first time that the intracellular labile iron pool can be targeted with a translational imaging strategy (i.e. PET) that is ideal for studying the biology of metastatic castration resistant prostate cancer. Our imaging technology and data provide the first evidence that clinically relevant mouse models of prostate cancer maintain high levels of iron. While these data suggest that iron could be targeted to for imaging to improve the detection of prostate cancer disease burden—itself a major unmet clinical need—they also provide a clear scientific rationale for testing if therapies can be implemented to treat prostate cancer tumors by targeting iron. This is an entirely new approach for treating prostate cancer that is nevertheless well justified based on successful antimicrobial therapies. Lastly, our technology for imaging iron is a crucial advance for cell biology, and the scientific community is now poised to use imaging to study iron flux in normal cellular physiology, and potentially non-malignant human disorders.

What was the impact on other disciplines?

Our interest in developing new anti-cancer diagnostics and therapeutics by targeting the labile iron pool parallels other imaging and medicinal chemistry efforts targeting LIP in non-malignant disorders. Other animal imaging approaches have relied on low resolution imaging modalities like bioluminescence, and some strategies require exotic genetic engineering of mice to express reporter proteins like luciferase. Our nuclear imaging strategy is much higher resolution, absolutely rather than semi-quantitative, and does not require any special genetic manipulation of mice. In this respect, our technology is poised to sensibly complement the ongoing imaging efforts in preclinical animal models of infectious disease by groups like Dr. Chris Chang's laboratory at UC Berkeley. The therapies that we are developing could potentially be applied to non-malignant neoplastic disorders, particularly those driven by mTORC1 or RAS. These include maladies like tuberous sclerosis complex and lymphangioleiomyomatosis, and RASopathies like neurofibromatosis.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Nothing to report

Changes in approach and reasons for change

We have de-emphasized the use of the ^{18}F-dioxolane (DXL) negative control compound during this project period. While the synthesis was straightforward and high yielding, we found that studying the biodistribution of ^{18}F-TRX after iron supplementation or withdrawal provided the necessary data to show that radiotracer accumulation in normal tissues was iron dependent. This experiment is also a better representation of naturally occurring changes in tissue concentrations of iron owing to environmental factors.

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

Project period one was generally very productive, and we did not encounter many challenges. We have remaining animal imaging experiments to execute using subcutaneous models and a PC3 dissemination model. We do not anticipate any challenges in setting up these animals, nor any difficulties with conducting the imaging studies. We were slightly delayed in executing these studies as we conducted some crucial animal biodistribution studies to show that ^{18}F-TRX biodistribution is iron dependent.

Changes that had a significant impact on expenditures

Nothing to report

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

Not applicable

Significant changes in use or care of vertebrate animals

Nothing to report. This project was approved by UCSF IACUC on August 21, 2018.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

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

  Journal publications.

  Muir RK, Zhao N, Wei J, Wang YH, Moroz A, Huang Y, Chen YC, Sriram R, Kurhanewicz J, Ruggero D, Renslo AR, Evans MJ. Measuring Dynamic Changes in the Labile Iron Pool

in Vivo with a Reactivity-Based Probe for Positron Emission Tomography. ACS Cent Sci. 2019 Apr 24; 5(4):727-736. PMID: 31041393., federal support was acknowledged

Books or other non-periodical, one-time publications. Nothing to report.

Other publications, conference papers and presentations.

Measuring dynamic changes in the labile iron pool with a reactivity-based probe for positron emission tomography. Poster abstract at the 2019 World Molecular Imaging Congress

Measuring dynamic changes in the labile iron pool with a reactivity-based probe for positron emission tomography. Poster presentation at the 2019 UCSF Radiology research symposium.

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

  Nothing to report

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

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

  Talukdar, P., Renslo, A.R., Blank, B.R., Muir, R.K., Evans, M.J. Trioxolane agents PCT/US2018/039768, published 03/2019

- **Other Products**

  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Davide Ruggero
Role: Principal Investigator
Nearest person month worked: 0.9
Contribution to project: Dr. Ruggero has been responsible for overall administration and guidance of the proposed research. He also reviewed the primary data, guided the overall direction of the research, assisted with trouble-shooting and alternative approaches, and coordinated with the collaborators. He has also coordinated the preparation, submission, and publication of abstracts and manuscripts reporting the results of the project.

Name: Crystal Conn
Role: Post-Doc
Nearest person month worked: 1.44
Contribution to project: Dr. Conn was responsible for breeding the colony of genetically engineered mice for imaging with ^{18}F-TRX and treatment with TRX-CBI as well as for the PET and biodistribution studies of 18F-TRX in genetically engineered mouse models.

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?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*