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TITLE: Radiolabeled PARP Inhibitors for Imaging and Targeted Radiotherapy of Prostate Cancer

PRINCIPAL INVESTIGATOR: Dr. Buck Rogers

CONTRACTING ORGANIZATION: Washington University, Saint Louis, MO

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14. ABSTRACT

The of this grant is to investigate a ^{77}Br-labeled poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor for Auger radiation targeted radiotherapy of mCRPC. PARP-1 is a nuclear enzyme which initiates DNA repair by binding to the sites of single- or double-strand breaks (SSB/DSB). The major goals of this reporting period were to 1.) Determine the biodistribution of [^{77}Br]WC-DZ-Br in PC-3 and IGR-CaP1 subcutaneous models, 2.) conduct single dose therapy studies of [^{77}Br]WC-DZ-Br in a metastatic model, 3.) conduct a multi-dose therapy study of [^{77}Br]WC-DZ-Br in a metastatic model. The major activities conducted in this reporting period were to evaluate [^{77}Br]WC-DZ-Br *in vivo* to determine its tissue localization and therapeutic efficacy after a single administration. The significant results of this reporting period were that [^{77}Br]WC-DZ-Br had good, specific uptake in PC-3 and IGR-CaP1 tumors at 4 h after injection and this uptake was higher than all other tissues except for the liver. In addition, in the metastatic IGR-CaP1 model, injection of [^{77}Br]WC-DZ-Br showed a trend of increasing survival, but this did not reach significance.

15. SUBJECT TERMS
PARP inhibitors, Auger radiation, bromine-77, PET imaging, DNA damage

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1. Introduction

Metastatic, castration-resistant prostate cancer (mCRPC) is a highly lethal disease with no curative therapeutic options. Targeted radiotherapy appears to be a promising approach for mCRPC. Alpha radiation therapy using $^{223}RaCl_2$ has shown a survival advantage in this patient population when compared to placebo, even though the radiation is targeted to the tumor microenvironment and not tumor cells themselves. Auger radiation is also highly cytotoxic, but the radiation decay must occur near the DNA of the targeted cell to be effective. This feature also makes Auger therapy attractive because there is little cytotoxic effect in cells that do not accumulate the radioactivity in the nucleus, leading to less overall toxicity. A major obstacle for Auger radiation therapy has been the lack of an appropriate targeting vehicle to deliver radiation efficiently into the cell nucleus. The **subject** of this grant is to investigate a ^{77}Br-labeled poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor for Auger radiation targeted radiotherapy of mCRPC. PARP-1 is a nuclear enzyme which initiates DNA repair by binding to the sites of single- or double-strand breaks (SSB/DSB). PARP-1 is overexpressed in many cancers including mCRPC, but not in normal tissues, and may therefore serve as a target for nuclear imaging as well as Auger radiation radiotherapy. We have identified a PARP-1 inhibitor (WC-DZ-Br) suitable for labeling with the Auger isotope ^{77}Br. [^{77}Br]WC-DZ-Br has high affinity for PARP-1 and high PARP-1 specific uptake in prostate cancer cells. The purpose of the research is to evaluate [^{77}Br]WC-DZ-Br in prostate cancer cells to determine its cytotoxicity and in mice bearing prostate cancer xenografts to determine therapeutic efficacy and safety.

2. Keywords: PARP inhibitors, Auger radiation, bromine-77, PET imaging, DNA damage

3. Accomplishments

What were the major goals of the project?
The major goals of this reporting period were to 1.) Determine the biodistribution of [^{77}Br]WC-DZ-Br in PC-3 and IGR-CaP1 subcutaneous models, 2.) conduct single dose therapy studies of [^{77}Br]WC-DZ-Br in a metastatic model, and 3.) conduct a multi-dose therapy study of [^{77}Br]WC-DZ-Br in a metastatic model. #1 was to be conducted in Months 16-20 to determine feasibility of the PET imaging studies and is 80% completed, #2 was to be conducted in Months 21-26 and is 100% completed, and #3 was to be conducted in Months 27-33 and is 0% completed.

What was accomplished under these goals?
The major activities accomplished in this reporting period were to evaluate [^{77}Br]WC-DZ-Br *in vivo* to determine its uptake in normal tissues and subcutaneous tumors and determine its therapeutic efficacy after a single administration in a metastatic model. The significant results of this reporting period were that [^{77}Br]WC-DZ-Br demonstrated specific uptake in PC-3 and IGR-CaP1 tumors 4 h after injection and that this uptake was higher than all other tissues except for the liver. In addition, uptake in other tissues was not PARP-1 specific. We began therapy studies using the IGR-CaP1 metastatic mouse model and found that although there is a trend toward improved survival upon treatment with [^{77}Br]WC-DZ-Br, this survival enhancement did not reach significance. These results are described in more detail below.

In the previous period, we performed a biodistribution evaluating uptake of [^{77}Br]WC-DZ-Br in mice bearing subcutaneous PC-3 or IGR-CaP1 tumors. In this study, PC-3 or IGR-CaP1 cells (1×10^7 cells in serum free DMEM) were injected to the right flank of female and male mice respectively. Once the tumors reached a volume of 100 mm^3, 370 kBq of [^{77}Br]Br-WC-DZ was injected intravenously and the biodistribution was evaluated at 4h (n = 5). In the blocking group, mice were pre-injected with olaparib (5 mg/kgbwt) and biodistribution was evaluated at 4h. The animals were sacrificed at each time point, their organs were harvested, and radioactivity was measured on a gamma counter. Tumor and organ uptake were analyzed and calculated as a percentage of injected dose per weight of tissue in grams (%ID/g) (**Figure 1A**). Tumor-to-organ ratios were calculated for each mouse based on %ID/g (**Figure 1B**). These data show that there was PARP-1 specific uptake in both PC-3 and IGR-CaP1 tumors, but not in other normal tissues. This is important in that this indicates that [^{77}Br]Br-WC-DZ should not induce toxicity in these tissues if uptake is not related to PARP-1. In addition, the tumor uptake was greater than in all other tissues except for the liver, indicating that [^{77}Br]Br-WC-DZ should have a favorable dosimetric profile. *In vivo* therapy studies were performed in mice bearing IGR-CaP1-mCherry-Luc metastatic tumors after intracardiac injection (**Figures 2, 3**). Mice (n = 5 per group) were injected into the left ventricle with IGR-CaP1-mCherry-Luc cells. One week later 1.8 mCi of [^{77}Br]WC-DZ-Br or saline was injected i.v. Tumor growth was monitored by bioluminescent imaging by imaging the ventral and dorsal side of the mice. Representative mice are shown in (**Figure 2**). Kaplan-Meier survival analysis shows that while there was a trend for increased survival for treated mice, it did not reach significance (**Figure 3**). Overall, these results are extremely important as they demonstrate good tumor uptake and low normal tissue uptake which would lead to a good dosimetry profile. In addition, the metastatic therapy results were encouraging although they did not reach significance. The next step is to repeat the single dose study in the metastatic tumor model as well as evaluate the multi-dose therapy in the metastatic model which we did not get to in year 3. Finally, we will perform combination therapy studies using [^{77}Br]WC-DZ-Br and docetaxel.

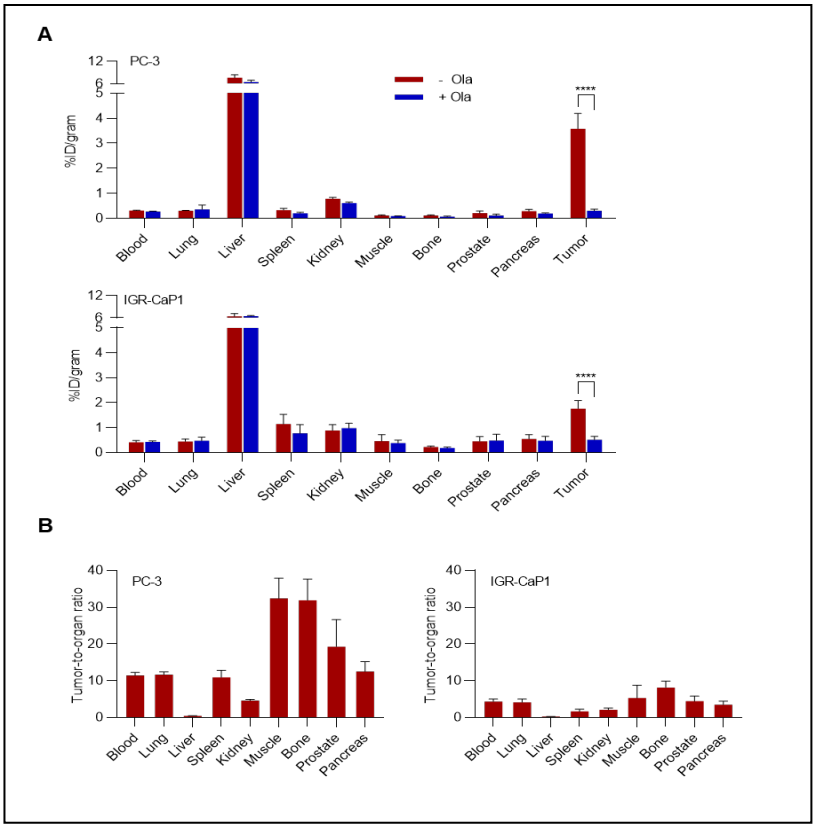

Figure 1. (A) **A,** Biodistribution of [^{77}Br]Br-WC-DZ (370 kBq) at 4h (+/- 5 mg/kgbwt olaparib block) in athymic nude mice (n = 5) bearing PC-3 and IGR-CaP1 tumors. The data represent the mean ± SD. **B,** Tumor-to-organ ratios of uptake of [^{77}Br]Br-WC-DZ (370 kBq) at 4h in PC-3 ad IGR-CaP1 tumors and selected organs of tumor-bearing mice.

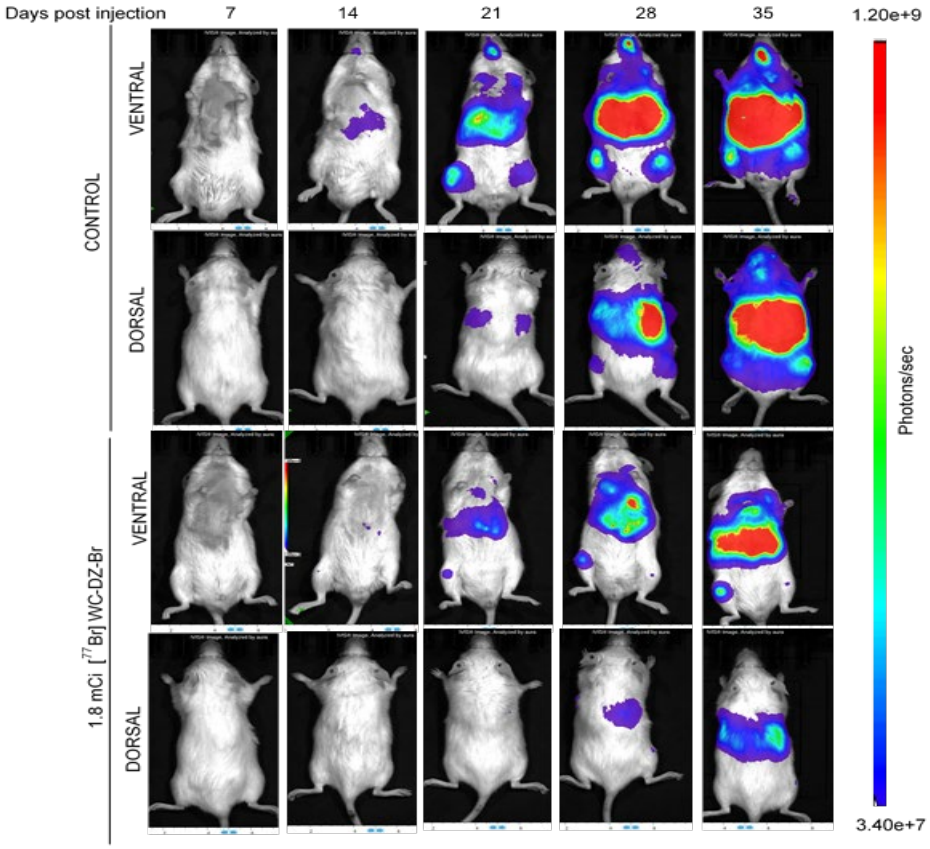

Figure 2. Bioluminescent imaging from a representative mouse bearing IGR-CaP1 metastatic tumors and treated with saline (control), top two columns or with 1.8 mCi of [^{77}Br]WC-DZ-Br, bottom two columns. A trend is seen of less tumor burden in mice treated with [^{77}Br]WC-DZ-Br.

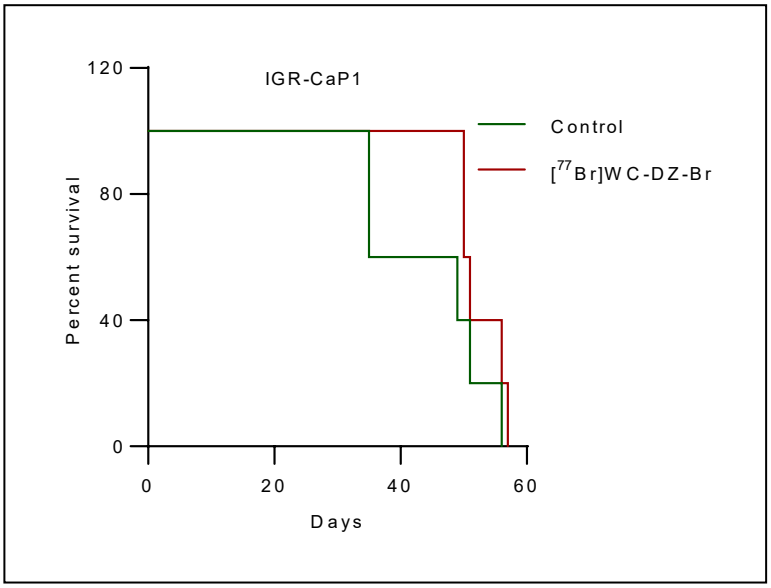

Figure 3. Kaplan–Meier survival curve of mice bearing IGR-CaP1 metastatic tumors and treated i.v. with either 1.8 mCi of [^{77}Br]WC-DZ-Br or saline (control). This shows that there was not a significantly enhance survival when mice were treated with 1.8 mCi of [^{77}Br]WC-DZ-Br compared to control, but there was a trend toward increased survival.

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?
The main goal of the next reporting period is to repeat the single dose study in the metastatic tumor model as well as evaluate the multi-dose therapy in the metastatic model which we did not get to in year 3. Finally, we will perform combination therapy studies using [^{77}Br]WC-DZ-Br and docetaxel.

4. Impact

What was the impact on the development of the principle(s) of the project?
The results from the first year show that a PARP inhibitor radiolabeled with Auger radioactivity can localize to the nucleus of prostate cancer cells, resulting in DNA damage that ultimately kills those cells. The second year has been highly important in that we have demonstrated therapeutic efficacy in two different prostate cancer models with no associated toxicities. The third year demonstrated good tumor uptake and low normal tissue uptake, which should translate to good dosimetry. There is a trend toward therapeutic efficacy in a metastatic model.

What was the impact on other disciplines?
The results presented here show the possibility of this type of radiation therapy *in vivo* for metastatic tumors. This may have an impact in the future in the development of these types of drugs for other types of cancer that metastasize.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?
These results may impact society by helping people understand the benefits of radiation for cancer treatment. Much of the general public is afraid of radiation, so showing how it can be used to help cure cancer and demonstrate its safety will help overcome these fears.

5. Changes/Problems

Changes in approach and reasons for change.
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them.
We did not perform the multi-dose studies of [^{77}Br]WC-DZ-Br in the metastatic model. We anticipate performing these studies in the no-cost extension year as well as performing combination therapy studies with docetaxel.

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.
Nothing to report.

Significant changes in use or care of human subjects.
Nothing to report.

Significant changes in use or care of vertebrate animals.
Nothing to report.

Significant changes in use of biohazards and/or select agents.
Nothing to report.

6. Products

Publications, conference papers, presentations.
Nothing to report.

Website(s) or other Internet site(s).
Nothing to report.

Technologies or techniques.
Nothing to report.

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: Buck Rogers
Project Role: PI
Nearest person month worked: 2
Contribution to Project: Oversaw all activities and planned experiments

Name: Dong Zhou
Project Role: Co-Investigator
Nearest person month worked: 2
Contribution to Project: Performed radiolabeling of PARP inhibitor

Name: Jinbin Xu
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Oversaw, performed, and planned in vitro experiments

Name: Sreeja Sreekumar
Project Role: Research Scientist
Nearest person month worked: 3
Contribution to Project: Performed in vivo experiments

Name: Huifangjie Li
Project Role: Research Technician
Nearest person month worked: 6
Contribution to Project: Performed in vitro experiments

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

Other support changes for Dr. Rogers:
Previous grants that closed: NIH- Development of Superior Chelation Chemistry for ^{89}Zr-ImmunoPET Imaging

Grants now active: NIH-Imaging and Targeted Auger Radiotherapy of High-Grade Glioma-Supplement

NIH-iSonogenetics for incisionless cell-type-specific neuromodulation of non-human primates

Other support changes for Dr. Zhou:
Previous grants that closed: None

Grants now active: NIH-Imaging and Targeted Auger Radiotherapy of High-Grade Glioma-Supplement

Other support changes for Dr. Xu:
Previous grants that closed: None

Grants now active:	NIH-Imaging and Targeted Auger Radiotherapy of High-Grade Glioma-Supplement
	NIH- Optimization of imaging mass cytometry, a single-cell spatial proteomics technology, for the study of Alzheimer disease
	NIH- Quantitative Endogenous MRI Imaging of Neuroinflammation in AD

What other organizations were involved as partners?
Nothing to report.

8. Special Reporting Requirements

Not applicable.

9. Appendices

Not applicable.