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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 maximum tolerated dose and toxicity of [^{77}Br]WC-DZ-Br, 2.) Conduct single dose therapy studies of [^{77}Br]WC-DZ-Br in tumor bearing mice, 3.) determine the dosimetry of [^{77}Br]WC-DZ-Br in normal mice, and 4.) Start therapy 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 therapeutic efficacy and toxicity after a single administration. The significant results of this reporting period were that [^{77}Br]WC-DZ-Br was relatively non-toxic in mice and that it demonstrated significant tumor growth delay in mice bearing subcutaneous PC-3 and IGR-CaP1 tumors. In addition, we have made progress in establishing the metatstatic IGR-CaP1 model.

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 maximum tolerated dose and toxicity of [^{77}Br]WC-DZ-Br, 2.) Conduct single dose therapy studies of [^{77}Br]WC-DZ-Br in tumor bearing mice, 3.) determine the dosimetry of [^{77}Br]WC-DZ-Br in normal mice, and 4.) Start therapy in a metastatic model. #1 was to be conducted in Months 11-15 and is 50% completed, #2 was to be conducted in Months 16-20 and is 100% completed, #3 was to be conducted in Months 16-20 and is 0% completed, and #4 was to begin in Month 21 and is 10% completed.

What was accomplished under these goals?
The major activities conducted in this reporting period were to evaluate [^{77}Br]WC-DZ-Br *in vivo* to determine its therapeutic efficacy and toxicity after a single administration. The specific objectives were to 1.) determine the MTD and toxicity of [^{77}Br]WC-DZ-Br, 2.) determine the therapeutic efficacy of [^{77}Br]WC-DZ-Br after a single administration, and 3.) determine the dosimetry of [^{77}Br]WC-DZ-Br. The significant results of this reporting period were that [^{77}Br]WC-DZ-Br was relatively non-toxic in mice and that it demonstrated significant tumor growth delay in mice bearing subcutaneous PC-3 and IGR-CaP1 tumors. In addition, we have made progress in establishing the metastatic IGR-CaP1 model. These results are described in more detail below.

It should be noted that we were still not able to create the ERG fusion cells and thus focused our efforts on evaluating the wild-type PC-3 and IGR-CaP1 cells.

In the previous period, we performed an MTD study in which it was found that 1.8 mCi of [^{77}Br]WC-DZ-Br could be given safely to mice. In this study, [^{77}Br]WC-DZ-Br was injected into normal, male mice and serum collected one and fourteen days later and compared to serum from mice injected with vehicle control. Day 1 data shows that there was not an increase in alanine transaminase (ALT) (*65 u/L vs. 59 u/L*), aspartate transaminase (AST) (162 *u/L vs 142 u/L*), alkaline phosphatase (*164 u/L vs. 166 u/L*), total bilirubin (*0.13 mg/dL vs. 0.09 mg/dL*), or total protein (*4.9 g/dL vs. 4.9 g/dL*). The same was true for day 14. In addition, tumor bearing mice did not show a decrease in body weight (**Figures 1C and 2C**). However, it should be noted that the MTD was not reached, so it may be possible to go to even higher radioactive doses with minimal to limited toxicity. This could result in even greater therapeutic efficacy. *In vivo* therapy studies were performed in mice bearing PC-3 (**Figure 1**) and IGR-CaP1 (**Figure 2**) tumors. Mice were implanted subcutaneously with cells and then administered 1.3 mCi (PC-3 tumors) or 1.8 mCi (IGR-CaP1 tumors) of [^{77}Br]WC-DZ-Br i.v. when the tumors reached ~175-300 mm^3. Tumors were measured with calipers twice per week and the data plotted as tumor volume versus time. **Figure 1A** shows the mean tumor volume (n = 7) of PC-3 tumors over time and shows that administration of [^{77}Br]WC-DZ-Br significantly inhibited tumor growth ($P < 0.0001$). **Figure 1B** shows progression of tumor volume for individual mice. There was not a drop in mouse body weight upon administration of [^{77}Br]WC-DZ-Br as observed in **Figure 1C**. Kaplan-Meier survival analysis shows a significant increase ($P < 0.0006$) in survival for treated mice (**Figure 1D**). Similar results were observed when we evaluated [^{77}Br]WC-DZ-Br in the IGR-CaP1 tumor model (n = 5) (**Figure 2A-D**). Overall, these results are extremely important as they demonstrate the overall concept of *in vivo* therapeutic efficacy using a radiolabeled PARP-1 inhibitor while being relatively non-toxic. The next step is to evaluate this in a metastatic tumor model. In this regard, **Figure 3** shows metastases of IGR-CaP1-mCherry-Luc cells three weeks after cardiac injection. This shows metastatic lesions in the lung, liver, and bone. This model will be used in the final year of the grant to determine if [^{77}Br]WC-DZ-Br will have therapeutic efficacy against these metastatic lesions.

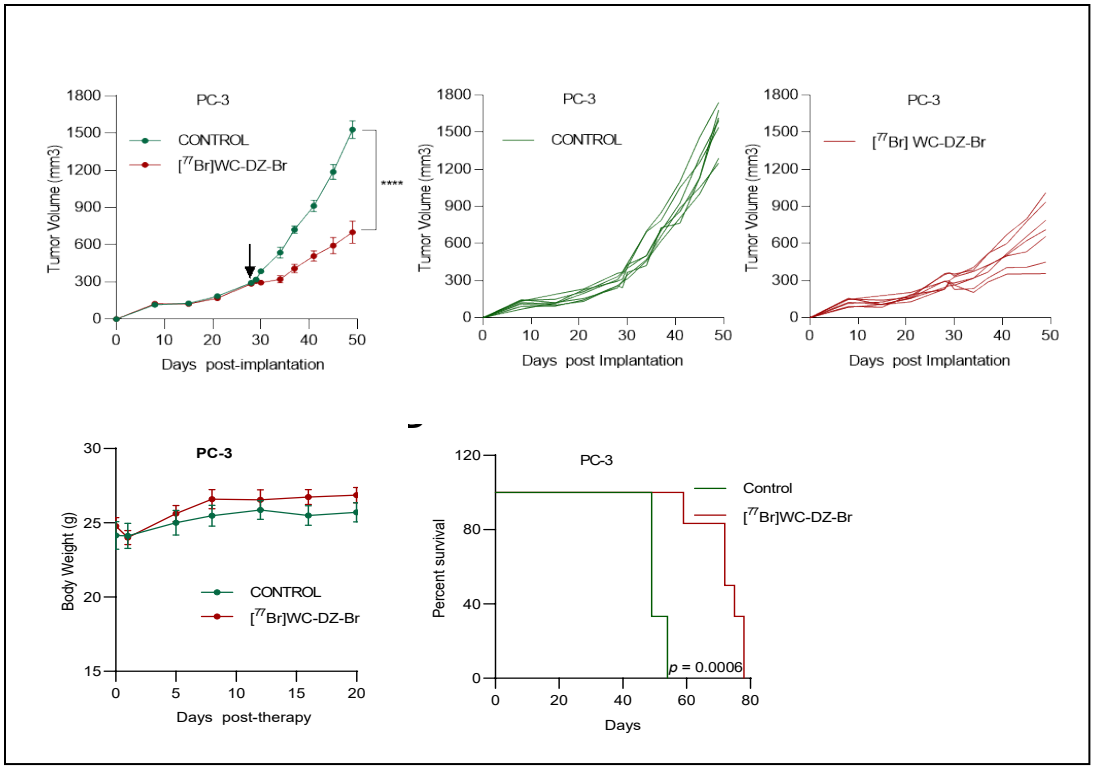

Figure 1. (A) Mean tumor volume (±SEM) in athymic nude mice bearing PC-3 xenograft after treatment with 1.3 mCi of [^{77}Br] WC-DZ-Br (n = 7). Control group received saline. The arrow indicates the day of treatment. Asterisks represent significant difference of the treatment group relative to control. ****P<0.0001. (B) Tumor volume progression for individual mice in each treatment group are shown. (C) Body weight (g) change in mice after receiving saline and [^{77}Br] WC-DZ-Br treatment. Body weight data are represented as the group mean ± SEM. (D) Kaplan–Meier survival study of PC-3 tumor implanted mice showed improved survival with [^{77}Br] WC-DZ-Br treatment compared to control (*P =0.0006).*

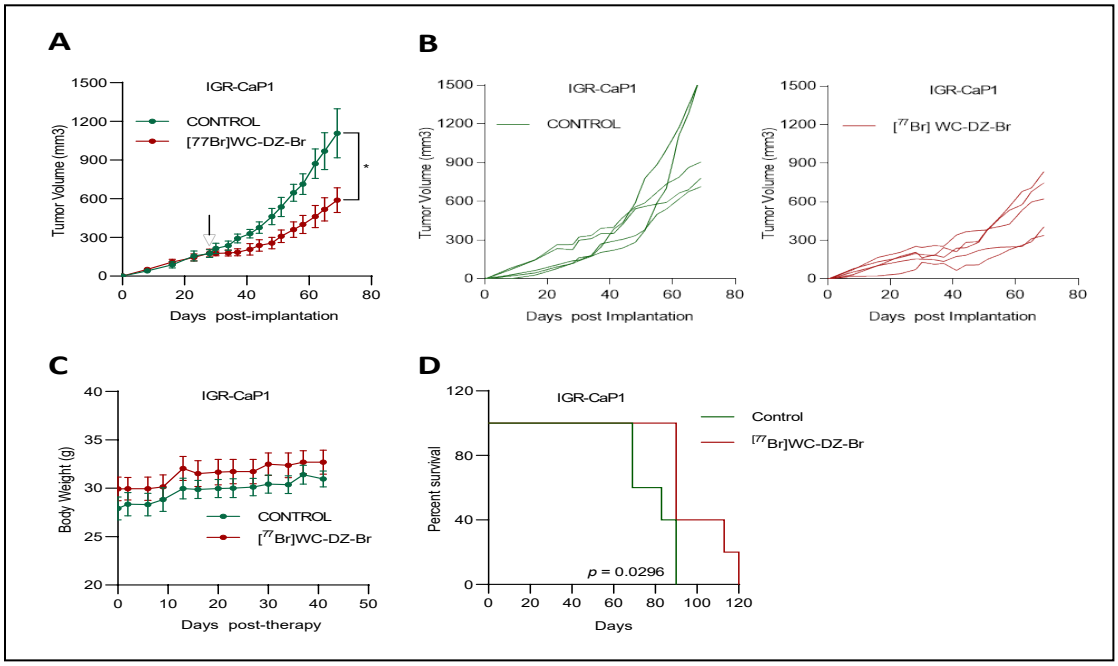

Figure 2. (A) Mean tumor volume (±SEM) in athymic nude mice bearing IGR-CaP1 xenograft after treatment with 1.8 mCi of [^{77}Br] WC-DZ-Br (n = 5). Control group received saline. The arrow indicates the day of treatment. Asterisks represent significant difference of the treatment group relative to control. *P<0.05. (B) Tumor volume progression for individual mice in each treatment group are shown. (C) Body weight (g) change in mice after receiving saline and and [^{77}Br] WC-DZ-Br treatment. Body weight data are represented as the group mean ± SEM. (D) Kaplan–Meier survival study of IGR-CaP1 tumor implanted mice showed improved survival with [^{77}Br] WC-DZ-Br treatment compared to control (*P =0.0296).*

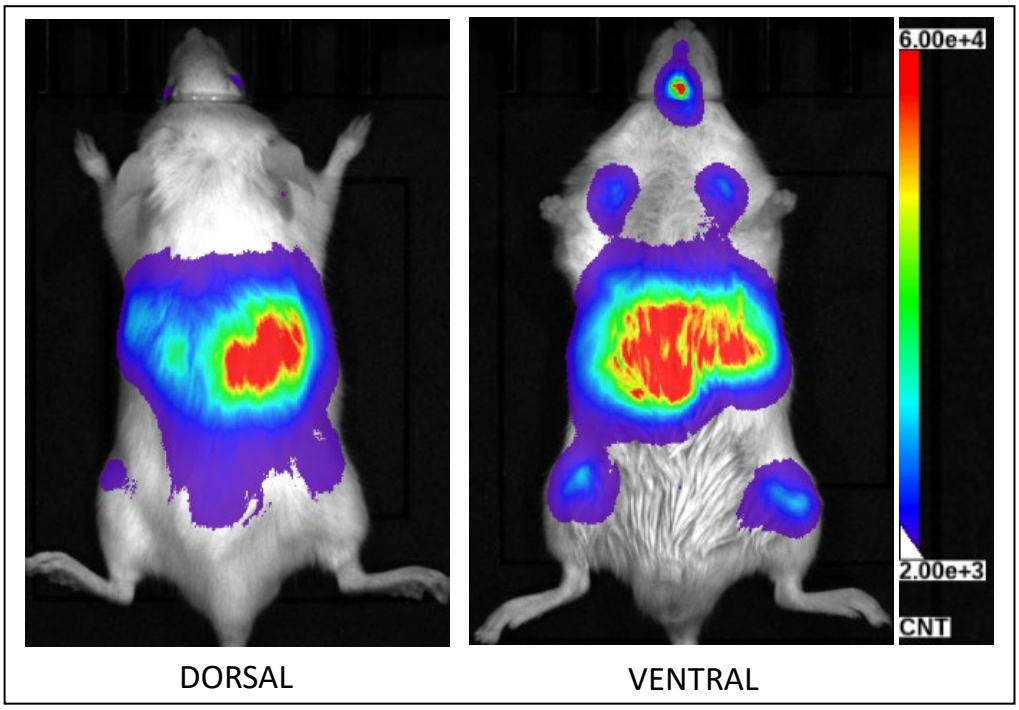

Figure 3. *In vivo* bioluminescence imaging showing signals detected after 3 weeks of injection of IGRCaP1-mCherry Luc cells to the left cardiac ventricle of NOD scid gamma (NSG) mice indicating metastasis .

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 demonstrate the therapeutic efficacy of [^{77}Br]WC-DZ-Br in a model of metastatic prostate cancer using both single and multiple doses. This will then be extended into investigating combination therapies with docetaxel. The radiation dosimetry will be evaluated in normal mice to determine the radiation dose delivered to normal tissues as was supposed to happen at the end of Year 2.

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.

What was the impact on other disciplines?
The results presented here show the efficacy of this type of radiation therapy *in vivo* and how it is relatively non-toxic to normal organs. This may have an impact in the future in the development of these types of drugs.

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.
As mentioned above we did not produce the ERG fusion cell lines. We will continue to troubleshoot this, but will simultaneously focus on therapy studies using the wild-type cells. In addition, due to COVID-19 delays and our focus on determining the therapeutic efficacy of [^{77}Br]WC-DZ-Br *in vivo*, we did not perform the dosimetry studies on [^{77}Br]WC-DZ-Br. We anticipate performing these studies in Year 3.

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: 9
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 a rapid method for imaging regional ventilation in small animals without contrast agents

NIH-Novel Bifunctional Chemical Agents as Theranostic Tools for Amyloid Diseases

Grants now active: NIH- Development of Superior Chelation Chemistry for ^{89}Zr-ImmunoPET Imaging

NIH- Molecular Imaging CCR2 Lung Inflammation and Fibrosis

Other support changes for Dr. Zhou:
Previous grants that closed: NIH- Understanding Sex Disparities in Gliomas through Sex Differences in

Mitochondrial Activity

Siteman Cancer Center- Imaging PARP levels to predict DNA-damaging agent treatment responses in pancreatic cancer

Grants now active: No new grants started

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

Grants now active: NIH- Dissect the mechanisms of selective regional vulnerability in Lewy Body Dementias via comparative snRNA-seq analysis

NIH- Define molecular events driving selective neuronal death in multiple neurodegenerative diseases by snRNA-seq.

NIH- Diffusion Basis Spectrum Imaging for Measurement of Neuroinflammation in Parkinson Disease.

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

8. Special Reporting Requirements

Not applicable.

9. Appendices

Not applicable.