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

PRINCIPAL INVESTIGATOR: Dr. Buck Rogers

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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 cytotoxicity of [^{77}Br]WC-DZ-Br and 2.) Conduct in vitro assays to determine [^{77}Br]WC-DZ-Br localization and DNA damage and 3.) start maximum tolerated dose (MTD) studies in mice. The major activities conducted in this reporting period were to radiolabel WC-BZ-Br with bromine-77 (^{77}Br) and determine its cytotoxicity in prostate cancer cells and determine its ability to cause DNA damage. The specific objectives were to 1.) create ERG fusion cells, 2.) determine cytotoxicity in these and wild-type cells, and 3.) determine DNA damage after addition of radioactivity. The significant results of this reporting period are that [^{77}Br]WC-DZ-Br was highly cytotoxic in wild-type prostate cancer cells and that significant DNA damage was reported using three different markers. It should be noted that we ran into technical issues trying to create the ERG fusion cells. Thus at this point all of the data presented is in wild-type cells.

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 cytotoxicity of [^{77}Br]WC-DZ-Br and 2.) Conduct in vitro assays to determine [^{77}Br]WC-DZ-Br localization and DNA damage and 3.) start maximum tolerated dose (MTD) studies in mice. #1 was to be conducted in Months 1-8 and is 50% completed, #2 was to be completed in Months 1-10 and is 50% completed and #3 was to begin in Month 11 but has been delayed due to coronavirus.

What was accomplished under these goals?
The major activities conducted in this reporting period were to radiolabel WC-BZ-Br with bromine-77 (77Br) and determine its cytotoxicity in prostate cancer cells and determine its ability to cause DNA damage. The specific objectives were to 1.) create ERG fusion cells, 2.) determine cytotoxicity in these and wild-type cells, and 3.) determine DNA damage after addition of radioactivity. The significant results of this reporting period are that [^{77}Br]WC-DZ-Br was highly cytotoxic in wild-type prostate cancer cells and that significant DNA damage was reported using three different markers. These results are described in more detail below.

It should be noted that we ran into technical issues trying to create the ERG fusion cells. Thus at this point all of the data presented is in wild-type cells. We have begun to troubleshoot the creation of these cells and anticipate having them soon.

Since the start of the grant we have increased the radiolabeling efficiency by increasing the radioactive yield from **40%** to **90%**. This allows us to optimize its use in cell culture and animal experiments. A comet assay was performed in PC-3 and IGR-CaP1 cells with [^{77}Br]WC-DZ-Br for 4 hours (**Figure 1**). The amount of DNA damage is expressed as the % of DNA in the tail (olive moment) and is 9.7 ± 3.8 and 8.7 ± 3.2 in non-treated PC-3 and IGR-CaP1, respectively compared to 59.4 ± 17.3 and 74.7 ± 6.7 for treated cells. This shows direct DNA damage when using [^{77}Br]WC-DZ-Br and is an indirect measure of the DNA localization of [^{77}Br]WC-DZ-Br since this is required for DNA damage by Auger-emitters. **Figure 2** shows that incubation of [^{77}Br]WC-DZ-Br with IGR-CaP1 cells (Left Panel) and PC-3 cells (Right Panel) results in recruitment of 53BP1, a protein invovled in the DNA damage response, and increases over the course of incubation. The formation of 53BP1 foci is quantitated in **Figure 3**. **Figure 4** shows that incubation of [^{77}Br]WC-DZ-Br with IGR-CaP1 cells (Left Panel) and PC-3 cells (Right Panel) results in formation of γH2AX foci, a marker of DNA double strand breaks, and increases over the course of incubation. The formation of γH2AX foci is quantitated in **Figure 5**. Increase in PARP expression is shown in **Figure 6** after exposure to [^{77}Br]WC-DZ-Br. We have shown significant cell killing of both PC-3 and IGR-CaP1 cells at 5 days with [^{77}Br]WC-DZ-Br (**Figure 7**) with IC_{50} values of 0.12 nM and 0.48 nM, respectively. By comparison the non-radioactive PARP-1 inhibitor olaparib did not show significant cell killing in either cell line, while the niraparib inhibitor had IC_{50} values of 1.2 μM and 3.4 μM in PC-3 and IGR-CaP1 cells respectively and the non-radioactive WC-DZ-Br had IC50 values of 1.2 μM and 16.8 μM, respectively. This data demonstrates the much greater cytotoxicity of the radioactive PARP-1 inhibitor compared to the non-radioactive PARP-1 inhibitors. In addition, a clonogenic assay demonstrated significant cell killing of IGR-CaP1 cells with [^{77}Br]WC-DZ-Br.

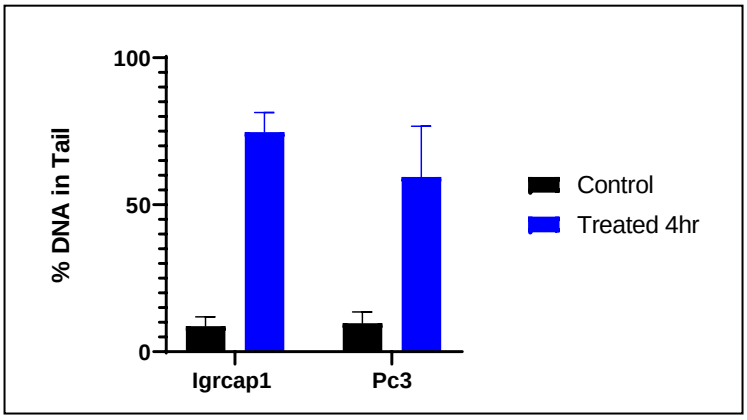


Figure 1. Comet assay showing the level of DNA damage in untreated PC-3 and IGR-CaP1 cells and cells treated with [^{77}Br]WC-DZ-Br for four hours. The bars represent the average %DNA in the tail of 17-20 cells +/- standard deviation.

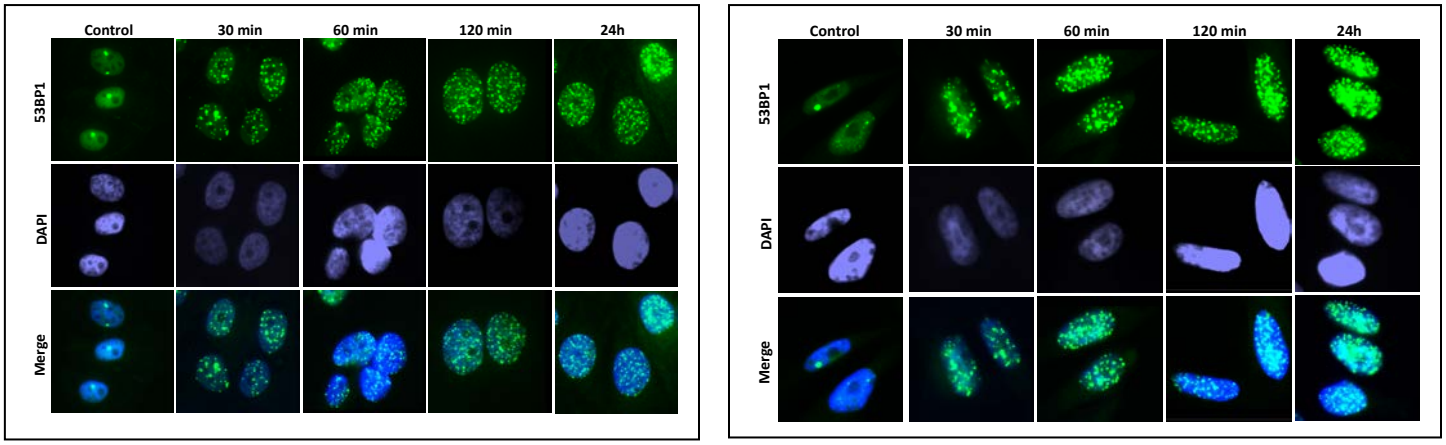


Figure 2. Immunofluorescence of 53BP1 foci after exposure of IGR-CaP1 cells (Left Panel) or PC-3 cells (right panel) to [^{77}Br]Wc-DZ-Br for various amounts of time. Top row-53BP1, middle-DAPI stain, lower-merged images.

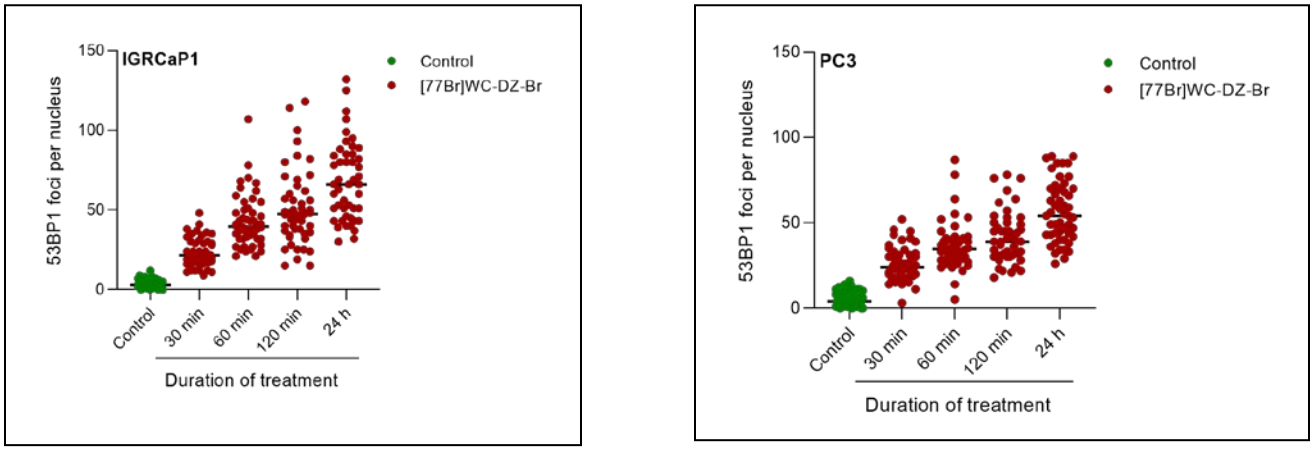


Figure 3. Quantitation of 53BP1 foci in IGR-CaP1 cells (Left Panel) and PC-3 cells (Right Panel) shows an increase in foci per cell over time.

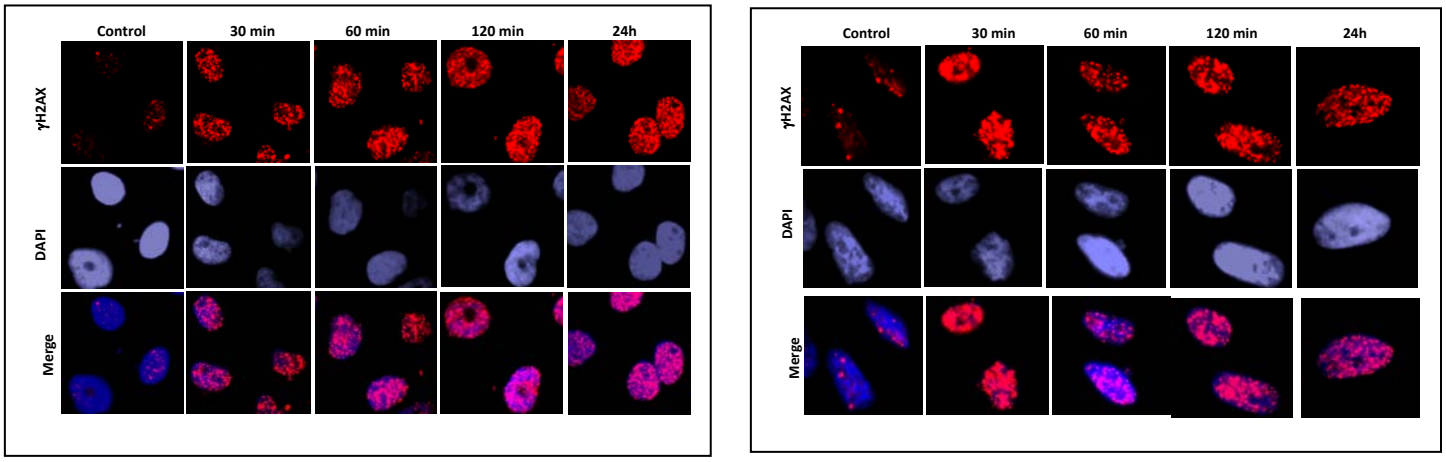
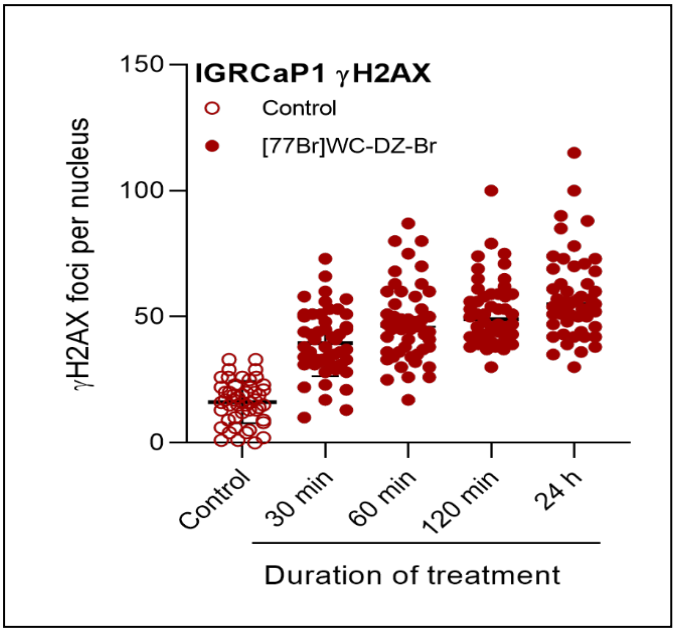


Figure 4. Immunofluorescence of γH2AX foci after exposure of IGR-CaP1 cells (Left Panel) or PC-3 cells (right panel) to [^{77}Br]Wc-DZ-Br for various amounts of time. Top row-γH2AX, middle-DAPI stain, lower-merged images.



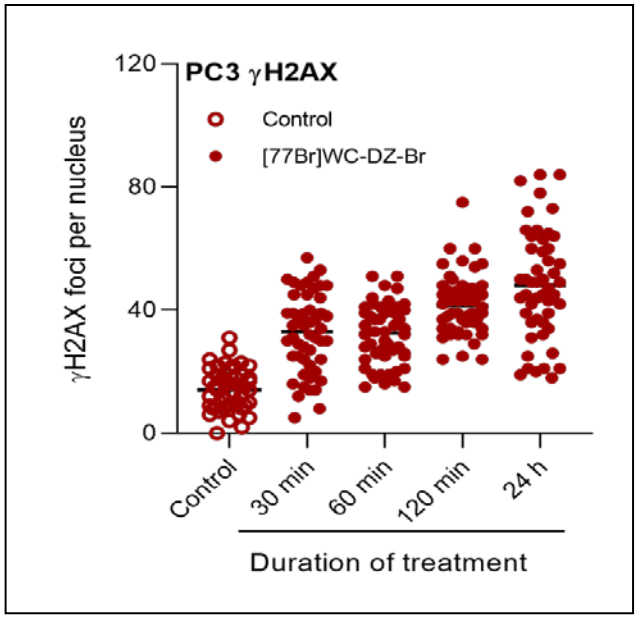


Figure 5. Quantitation of γH2AX foci in IGR-CaP1 cells (Left Panel) and PC-3 cells (Right Panel) shows an increase in foci per cell over time.

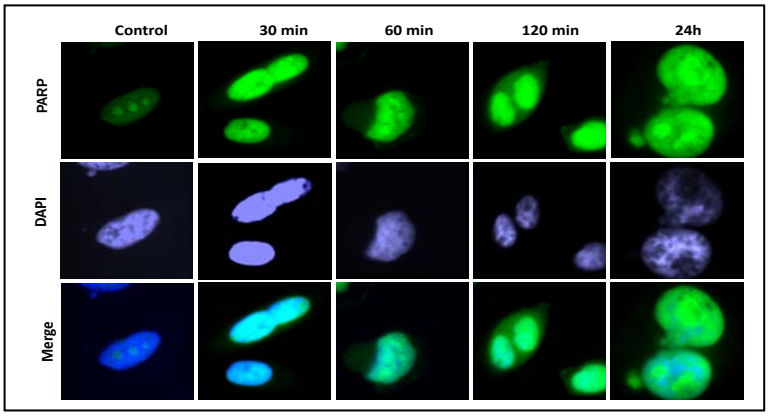


Figure 6. Immunofluorescence shows increase in PARP expression in PC-3 cells after treatment with [^{77}Br]WC-DZ-Br.

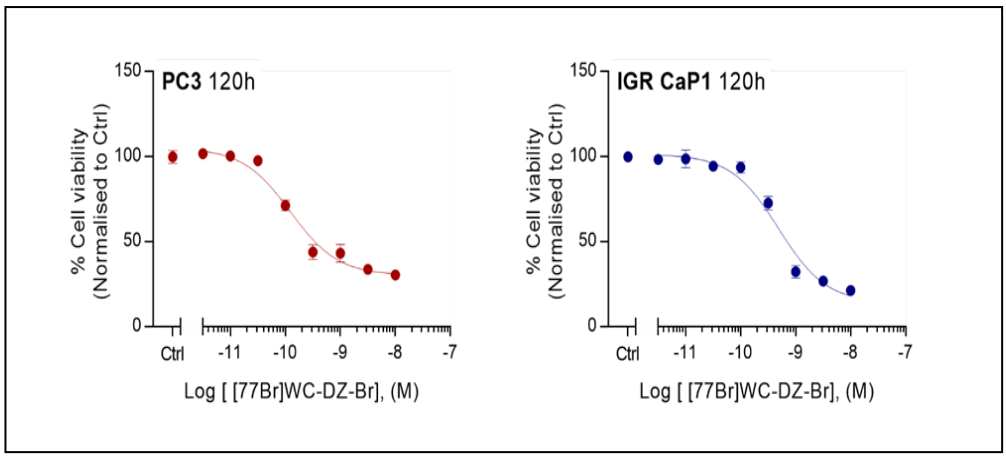


Figure 7. Dose-dependent killing of PC-3 cells (left) and IGR-CaP1 cells (right) with [^{77}Br]WC-DZ-Br after 5 days.

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 plan on trying to make the ERG fusion cells so that we can determine if this fusion leads to more sensitivity to [^{77}Br]WC-DZ-Br therapy. We anticipate we will follow the statement of work as planned. We will determine the maximum tolerated dose of [^{77}Br]WC-DZ-Br. We will then determine the therapeutic efficacy of [^{77}Br]WC-DZ-Br in mice bearing subcutaneous PC-3 tumors. In addition, the radiation dosimetry will be evaluated in normal mice to determine the radiation dose delivered to normal tissues.

4. Impact

What was the impact on the development of the principle(s) of the project?
The results thus far show that a PARP inhibitor radiolabeled with Auger radioactivity can localize to the nucleus of prostate cancer cells. This results in DNA damage that ultimately kills those cells.

What was the impact on other disciplines?
The results presented here show the efficacy of this type of radiation therapy and how it is more effective than the non-radioactive counterpart. 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 or main problem was production of the ERG fusion cell lines. We believe we were using the incorrect transfection conditions when attempting this. We will be optimizing this and intend to have the cells during the next reporting period.

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: Cedric Mpoy
Project Role: Research Technician
Nearest person month worked: 3
Contribution to Project: Performed in vitro experiments

Name: Sreeja Sreekumar
Project Role: Research Scientist
Nearest person month worked: 8
Contribution to Project: Performed in vitro 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: ICTS- Development of a novel theranostic agent for breast cancer imaging and treatment

Anonymous- Treatment of Metastatic Prostate Cancer with Radiolabeled PARP Inhibitors

Siteman Cancer Center-Radiolabeled PARP Inhibitors for Prostate Cancer Treatment

NIH- Development of a rapid method for imaging regional ventilation in small animals without contrast agents

Grants now active: NIH- The Radium-223 Combination Therapy Space; Improving Response and Clarifying Toxicities

NIH- Imaging and Targeted Auger Radiotherapy of High-Grade Glioma

NIH- Small Molecule GPCR Ligands for Oncologic Imaging

NIH- The Impact of Macrophage Origin on the Pathogenesis and Treatment Resistance of Pancreatic Cancer

Other support changes for Dr. Zhou:

Previous grants that closed: Anonymous- Treatment of Metastatic Prostate Cancer with Radiolabeled PARP Inhibitors

Siteman Cancer Center-Radiolabeled PARP Inhibitors for Prostate Cancer Treatment

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

NIH-Imaging Biomarkers Quantification and Standardization Core

Grants now active: NIH- Washington University Co-Clinical Imaging Research

NIH- Imaging and Targeted Auger Radiotherapy of High-Grade Glioma

NIH- Small Molecule GPCR Ligands for Oncologic Imaging

NIH- Understanding Sex Disparities in Gliomas through Sex Differences in Mitochondrial Activity

NIH- Neuroinflammatory Biomarkers for Nigrostriatal Injury

Other support changes for Dr. Xu:
Previous grants that closed: Anonymous- Treatment of Metastatic Prostate Cancer with Radiolabeled PARP Inhibitors

Siteman Cancer Center-Radiolabeled PARP Inhibitors for Prostate Cancer Treatment

NIH- Dominantly Inherited Alzheimer Network Trials Unit-Adaptive Prevention Trial

Bright Focus Foundation-PET-MRI Imaging of White Matter Damages and Inflammation in AD

Banner Alzheimer's Institute-Amyloid PET as a Biomarker for White Matter Integrity in Alzheimer Disease

Grants now active: NIH- Dominantly Inherited Alzheimer Network

NIH- Imaging and Targeted Auger Radiotherapy of High-Grade Glioma

NIH- Small Molecule GPCR Ligands for Oncologic Imaging

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

8. Special Reporting Requirements

Not applicable.

9. Appendices

Not applicable.