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TITLE: Development of a Radioligand to Detect Glucocorticoid Receptor Expression in Enzalutamide-Resistant Prostate Cancer with Positron Emission Tomography

PRINCIPAL INVESTIGATOR: Dr. Michael Evans

CONTRACTING ORGANIZATION:
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Dr. Michael Evans

E-Mail:

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University of California, San Francisco
1855 Folsom St
San Francisco, CA 94103-4249

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this project is to develop radioligands to measure glucocorticoid receptor (GR) expression in enzalutamide resistant prostate cancer. GR was recently identified in a preclinical model of acquired resistance to enzalutamide, and its expression was confirmed in drug resistant patient specimens. As spontaneous GR overexpression is one of several potential mechanisms of resistance to enzalutamide, we are leading an effort to develop a translational imaging assay to detect those patients with resistance due to GR overexpression. A small library of two steroidal and one non-steroidal radioligand to GR have been developed. All three are potent and selective GR ligands in vitro. Moreover, we have shown that one steroidal radioligand is stable in vivo and can specifically target GR in normal and cancerous tissues.

15. SUBJECT TERMS

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1. Introduction: The purpose of this project is to develop radioligands to measure glucocorticoid receptor (GR) expression in enzalutamide resistant prostate cancer. GR was recently identified in a preclinical model of acquired resistance to enzalutamide, and its expression was confirmed in drug resistant patient specimens. As spontaneous GR overexpression is one of several potential mechanisms of resistance to enzalutamide, we are leading an effort to develop a translational imaging assay to detect those patients with resistance due to GR overexpression. A small library of two steroidal and one non-steroidal radioligand to GR have been developed. All three are potent and selective GR ligands in vitro. Moreover, we have shown that one steroidal radioligand is stable in vivo and can specifically target GR in normal and cancerous tissues. Moving forward, we are summarizing the results for disclosure, as well as continuing to study the non-steroidal GR radioligand, which we anticipate to have more favorable pharmacokinetics in vivo.

2. Keywords: Enzalutamide, castration resistant prostate cancer, glucocorticoid receptor. PET, molecular imaging

3. Accomplishments:

Goals: The goals of this proposal are to (1) develop fluorine-18 labeled small molecule radioligands to detect GR expression in cancer cells, and (2) to assess their pharmacology in vitro and in vivo with small animal PET/CT.

Accomplishments: Since the start of this funding period, we have synthesized three radioligands to GR in high radiochemical yield and purity. The structures of the molecules are outlined in Figure 1. The affinity of each of the molecules was tested for human full length GR in a cellular competition assay with ^{3}H-dexamethasone, and all of the molecules have the low nM affinity required to be an effective radiotracer for PET (Table 1). Moreover, none of the molecules had affinity for other nuclear hormone receptors in the same family that was within 100 fold of GR, a cutoff that we established for ourselves in the original grant application.

We have studied each of the molecules in vivo as well. GR01 was shown to be unstable in vivo in immunocompetent mice, and substantial radiodefluorination was observed with concomitant uptake of fluoride ion in the bone (Figure 2A). We abandoned this compound on that basis. GR02 is an aryl fluoride and we found it to be far more stable in vivo, as expected (Figure 2B). Moreover, GR02 bound specifically to GR in normal mouse tissues (e.g. the liver), as binding could be suppressed by pretreating immunocompetent Bl6 mice with RU486, a GR antagonist (30 mg/kg, daily oral gavage, see Figure 2B and 2C). In addition, uptake of GR02 was higher in the normal tissues of adrenalectomized Bl6 mice (Figure 2D), a result highly consistent with specific binding to GR in vivo.

We next tested whether GR02 could detect GR expression in human prostate cancer xenografts. We previously conducted saturation binding assays and showed that prostate cancer models like PC3 and DU145 have supraphysiogic expression of GR compared to normal tissues (data not shown). Male nu/nu mice were inoculated with subcutaneous PC3 xenografts in the flank, and were treated with vehicle or RU486 for three days prior to the imaging experiment. The biodistribution of GR02 at 1 hour post injection showed that the activity in the tumor could be suppressed by RU486 to a statistically significant extent (Figure 3A).

Subcutaneous xenografts are idealized and convenient systems that do not necessarily reflect the vascularity of a tumor in humans. Therefore, we also tested the ability of GR02 to detect GR in PC3 xenografts grown within the renal capsule. As expected, the tumor uptake of the radiotracer was 10 fold higher in the more vascularized tumors (Figure 3B). Collectively, these data show that GR02 can specifically detect GR in normal and cancerous tissues with small animal PET/CT.

Training and professional development: The key personnel in this grant (Drs. Evans and Parker) have been working closely with Dr. VanBrocklin and other imaging experts at UCSF to better understand the theory and practice of fluorine-18 and carbon-11 radiochemistry. As a result, Drs. Evans and Parker are now very proficient in the design and execution of these radiochemical experiments, and approaching a level of expertise that will allow them to function independently. Dr. Parker, an organic chemist by training, has also been mentored by Dr. Evans in the pharmacological experiments required for preclinical radiotracer development.

As a part of professional development opportunities, these data have been presented as a poster by Dr. Evans at the Department of Defense IMPACT conference in Baltimore, MD. Moreover, Drs. Evans and Parker have presented these data internally at several symposia hosted by UCSF, including the Helen Diller Family Cancer Center annual retreat, the Department of Radiology and Biomedical Imaging annual research symposium, and the Program in Cancer Imaging and Therapy meeting at UCSF. These data were also presented at the Society of Nuclear Medicine and Molecular Imaging 2017 national meeting in Denver, CO.

Dissemination of results: The results have not yet been disclosed in peer reviewed literature. We plan to first publish the data with GR01 and GR02, and then submit a follow-up paper describing the synthesis and characterization of the non-steroidal radioligand. The data have been summarized in one published abstract from the SNMMI 2017 national meeting (Parker et al., J Nucl Med May 1, 2017 vol. 58 no. supplement 1 928).

Future plans: Moving forward, we will complete the analysis of GR02 by showing that the signal in tumors within the renal capsule can be competed with RU486. Moreover, we are developing a radiolabeled non-steroidal ligand that we expect to have better brain penetrance than GR02. This could expand the applications of the radioligand to stress related conditions that are driven by GR expression changes in the brain.

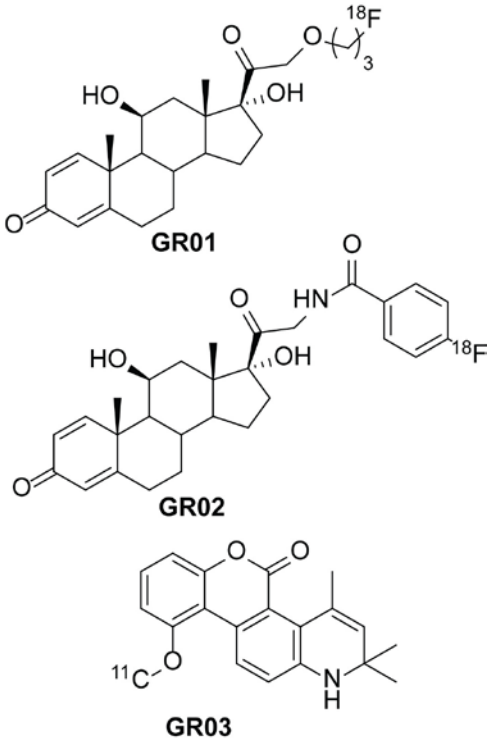

Figure 1. Structures of the new radioligands synthesized as part of year 1 of the funding period.

	K_i (nM)				
Entry	**GR**	**AR**	**PR**	**MR**	**ER**
GR01	3.8	1,460	1,060	876	1,113
GR02	2.3	584	718	1,500	984
GR03	15.9	2,201	893	1,236	784

Table 1. Ki values for the radioligands, determined with competition binding assays using ^{3}H-labeled steroids as references.

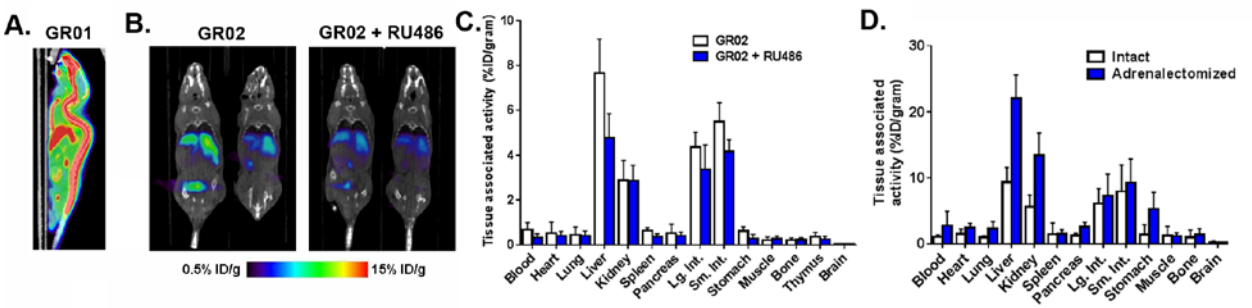

Figure 2. A. A representative PET image showing radiodefluorination of GR01 in normal black 6 mice 1 hour post injection. B. Representative PET/CT images showing that GR02 uptake in the liver of tumor naïve black 6 mice can be suppressed by pre-treatment with the GR antagonist RU486. The liver can be visualized in the center of the mouse's abdomen. C. Biodistribution data shows the blocking effect suggested by the PET data. D. Biodistribution data showing higher uptake of the radiotracer in normal tissues of adrenalectomized mice compared to normal black 6 mice with circulating corticosteroids.

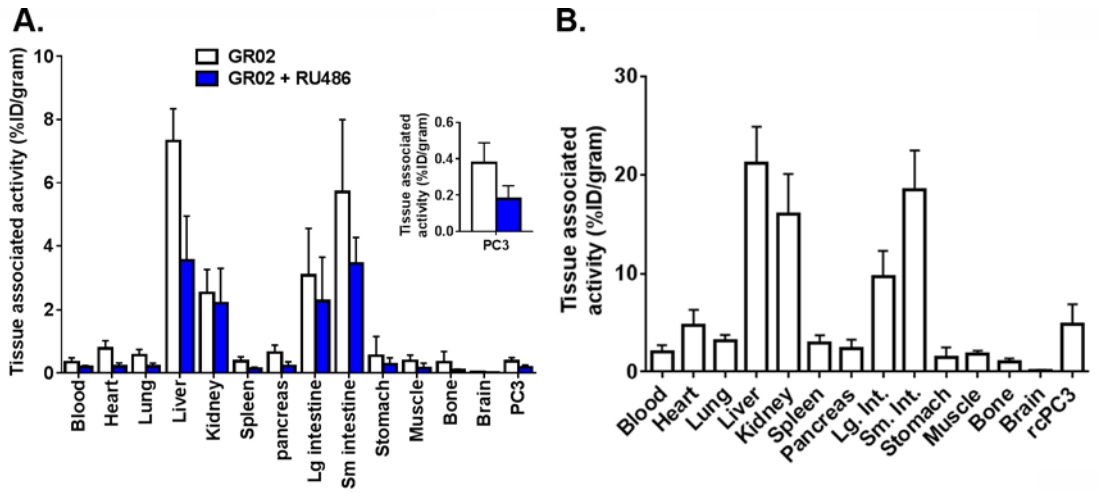

Figure 3. A. Biodistribution data in male nu/nu mice bearing subcutaneous PC3 tumors shows a blocking effect due to RU486 treatment in several normal mouse tissues, as well as in the PC3 cells (inset). B. Biodistribution data showing that GR02 uptake in a PC3 tumor within the renal capsule (rcPC3) is ten-fold higher than the uptake in subcutaneous PC3 tumors.

4. **Impact:**

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

Our data show for the first time that GR can be imaged in normal and prostate cancer tissues. This is significant, as it will allow the field to test in humans whether GR upregulation is (as expected) a dominant mechanism of resistance to enzalutamide compared to other mechanisms discovered in preclinical models (e.g, AR F876L, AR V7 splice variants, transdifferentiation to neuroendocrine prostate cancer). Moreover, our data showing that GR02 competes for GR binding with RU486 suggests that serial PET scanning can be used to interpret the pharmacodynamics effects of anti-corticosteroid therapy currently under clinical evaluation.

What was the impact on other disciplines?

A radiotracer to measure GR expression could be used to study the role of expression changes in other important cancers like breast and ovarian cancer. GR overexpression has been linked to chemoresistance in both cancers. Moreover, a radiotracer to study GR levels could be highly impactful to the neuroimaging field, as GR expression in the brain is involved in stress and depression.

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 and problems:

Nothing to report

6. Products:

Publications, conference papers, and presentations

Publications: nothing to report

Conference papers: Parker et al, J Nucl Med May 1, 2017 vol. 58 no. supplement 1 928

Presentations: Several poster presentations at internal research symposia hosted by UCSF.

Websites

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:	*Michael J. Evans*
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*0.56*
Contribution to Project:	Dr. Evans is responsible for overall project administration and coordination
Funding Support:	N/A
Name:	*Matthew Parker*
Project Role:	Post-Doctoral Researcher
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*10*
Contribution to Project:	Dr. Parker is responsible for the organic and radiochemistry, as well as the preclinical pharmacological assessment of new radiotracers.
Funding Support:	N/A

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

Yes, please see below.

Evans, Michael J.

Current

Title: 4R00CA172695-03 Noninvasive measurement of oncogenic signaling pathways with ^{89}Zr-transferrin **(PI: Evans)**
Time Commitment: 7.68 ca. mo
Supporting Agency: NIH/NCI

Grants Officer: Anne Menkens
Cancer Imaging Program
National Cancer Institute
Division of Cancer Treatment and Diagnosis
9609 Medical Center Drive
Bethesda, MD 20892-9725
240-276-6510
am187k@nih.gov

Performance Period: 4/15/2014 - 12/31/2016

Level of Funding: Annual Direct Costs $194,079

Project Goals: The aim of this proposal is to show that ^{89}Zr-transferrin measures PI3K pathway activity in glioblastoma.

Overlap: None.

Title: Exploiting the genetics of advanced prostate cancer for tumor detection and therapy with transferrin radionuclides **(PI: Evans)**

Time Commitment: 0.0 ca. mo

Supporting Agency: Prostate Cancer Foundation

Grants Officer: Audrey Gardner
Prostate Cancer Foundation
1250 Fourth Street
Santa Monica, CA 90401
310-570-4792
agardner@pcf.org

Performance Period: 5/20/2013-5/20/2017

Level of Funding: Annual Direct Costs $75,000

Project Goals: The aim of this proposal is to show that radiolabeled transferrin adducts can be used to image and treat prostate cancer harboring two abundant genetic lesions that promote transferrin uptake.

Overlap: None.

Title: 1R01CA176671-02 Annotating oncogene status with ^{89}Zr-transferrin **(PI: Evans, Lewis)**

Time Commitment: 1.2 ca. mo

Supporting Agency: NIH/NCI

Grants Officer: Anne Menkens
Cancer Imaging Program
National Cancer Institute
Division of Cancer Treatment and Diagnosis
9609 Medical Center Drive
Bethesda, MD 20892-9725
240-276-6510
am187k@nih.gov

Performance Period: 4/1/2014-3/31/2018

Level of Funding: Annual Direct Costs $100,006

Project Goals: The aim of this proposal is to show that ^{89}Zr-transferrin measures MYC status in prostate cancer and lymphoma, as well as PI3K pathway activity in prostate cancer. A first in man trial is planned for men with newly diagnosed prostate cancer.

Overlap: None.

New Award (this Technical Report is for this award)

Title:	W81XWH-15-1-0552 Development of a Radioligand to Detect Glucocorticoid Receptor Expression in Enzalutamide-Resistant Prostate Cancer with Positron Emission Tomography **(PI: Evans)**
Time Commitment:	0.56 ca. mo
Supporting Agency:	DOD
Grants Officer:	Joshua McKean Grant Specialist USAMRAA-Assistance Branch 4 820 Chandler Street, Fort Detrick, MD 21702 301-619-4046 Joshua.d.mckean3.civ@mail.mil
Performance Period:	9/30/2015-9/29/2017
Level of Funding:	Annual Direct Costs $115,494
Project Goals:	The central hypothesis of this grant is that treatment induced upregulation of GR expression (and activity) can be effectively measured with PET via a radioligand specifically targeting GR.
Specific Aims	**Specific Aim 1:** Synthesis and *in vitro* evaluation of the pharmacology of steroidal and non-steroidal 18F-radioligands to GR. **Specific Aim 2:** Proof of concept in vivo studies in animals bearing human prostate cancer models.
Overlap:	None.

New Award

Title:	Development of PET MR Imaging and Processing **(PI: Majumdar)**
Time Commitment:	0.64 ca. mo
Supporting Agency:	GE Healthcare
Grants Officer:	Yi Xia Senior Research Manager 9900 W Innovation Dr, Milwaukee, WI 53226
Performance Period:	06/01/2016 - 06/30/2019
Level of Funding:	Annual Direct Costs $405,649.00
Project Goals:	The aim of this project is to determine if hepatocellular carcinoma is detectable with 68Ga-citrate PET/MRI.
Overlap:	None.

New Award

Title:	Measuring MYC activity in double hit lymphoma with 68Ga-citrate (**PI: Evans)**
Time Commitment:	0.60 ca. mo
Supporting Agency:	UCSF Office of the Academic Senate
Grants Officer:	Kenneth Laslavic 500 Parnassus Avenue Room MUE-230 San Francisco, CA 94143
Performance Period:	02/01/2016 - 07/31/2017
Level of Funding:	Annual Direct Costs $73,131.00

Project Goals: The aim of this project is whether patients with double hit lymphoma harbor tumors with high avidity for 68Ga-citrate
Overlap: None.

New Award
Title: W81XWH-16-1-0469 Annotating MYC Status in Treatment-Resistant Metastatic Castration-Resistant Prostate Cancer with Gallium-68 Citrate PET **(PI: Aggarwal)**
Time Commitment: 0.60 ca. mo
Supporting Agency: DOD
Grants Officer: Ramachandran Arudchandran
USA Med Research Acq Activity
820 Chandler Street,
Fort Detrick, MD 21702
301-619-7099r
ramachandran.arudchandran.civ@mail.mil
Performance Period: 09/01/2016 - 08/31/2019
Level of Funding: Annual Direct Costs $81,832
Project Goals: The aim of this study is to use ^{68}Ga-citrate PET to identify tumors that harbor resistance to Abiraterone by upregulating MYC.
Overlap: None.

What other organizations were involved as partners?

Nothing to report