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TITLE: Multifunctional PSCA Antibody Fragments for PET and Optical Prostate Cancer Imaging

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Imaging remains a major unmet need in the management of prostate cancer. We are developing imaging probes based on engineered antibodies that recognize PSCA (prostate stem cell antigen), a cell surface protein highly expressed in prostate cancer. These engineered antibody fragments (cys-minibodies and cys-diabodies) can be labeled with radioisotopes for non-invasive PET imaging for use at multiple points in the prostate cancer treatment continuum, including staging at diagnosis, monitoring treatment, and re-staging at various points during management. Engineered fragments can also be labeled with fluorescent dyes for visual guidance in an intraoperative setting to ensure complete resection with negative margins. In this project, dually-labeled PSCA imaging agents are being developed that can be used for pre- and intra-operative detection of prostate cancer.
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1. INTRODUCTION
Imaging remains a major unmet need in the management of prostate cancer. We are developing imaging probes based on engineered antibodies that recognize PSCA (prostate stem cell antigen), a cell surface protein highly expressed in prostate cancer. These engineered antibody fragments (cys-minibodies and cys-diabodies) can be labeled with radioisotopes for non-invasive PET imaging for use at multiple points in the prostate cancer treatment continuum, including staging at diagnosis, monitoring treatment, and re-staging at various points during management. Engineered fragments can also be labeled with fluorescent dyes for visual guidance in an intraoperative setting to ensure complete resection with negative margins. In this project, dually-labeled PSCA imaging agents are being developed that can be used for pre- and intra-operative detection of prostate cancer.

2. KEYWORDS
Prostate cancer, imaging, antibody fragment, positron emission tomography, fluorescence imaging, PSCA

3. ACCOMPLISHMENTS
The major goals of the project are:

Specific Aim 1. Develop universal optimized cys-diabody and cys-minibody fragments against PSCA for PET imaging of prostate and pancreatic cancer

1) Major Task 1: Develop and evaluate cys-diabody and cys-minibody fragments (Year 1-2)

2) Major Task 2: Design, optimize and test multifunctional, F-18, and alternatively labeled antibody fragments (Year 2-3)

3) Major Task 3: New technologies (Year 3)

Specific Aim 2. Evaluate the ability of lead PSCA fragments to imaging prostate cancer in disease progression in xenograft and genetically engineered models of prostate cancer

4) Major Task 4: Image bone and lymph node in xenograft models

5) Major Task 5: Image transgenic mouse models

Specific Aim 3: Evaluate the ability of lead PSCA fragments to visualize prostate cancer “intraoperatively”.

6) Development and evaluation of singly labeled and optimized optical probes for surgery

7) Development of dual labeled probes for PET and optical imaging
What was accomplished under these goals:

Year 1 has focused on the development, production, and optimization of the PET imaging agents, on development of metastatic models for imaging, and in vivo fluorescent surgery experiments using singly labeled fragments.

Two closely-related sequence variants, A2 and A11 have been evaluated. Of note, a novel A11 cysteine minibody has been designed, produced, and purified, and this has been designated the lead candidate for singly and dually-labeled imaging agents. The protein has been radiolabeled with Zr-89 (site-specific) (Figure 1) and I-124 (Figure 2) for PET imaging studies.

Importantly, significant progress has been made ahead of schedule on production and evaluation of a dually-labeled PET/optical probe, as detailed in the abstract below, which was presented at the Antibody Technology Resource Center Symposium at UC San Francisco, October 2016.

Dual-modality immuno-PET/fluorescence imaging of prostate cancer using anti-PSCA A11 cysteine minibody

Wen-Ting Tsai1, Kirstin Zettlitz1, Richard Tavaré1, Felix Salazar1, Robert Reiter2, and Anna Wu1
1Crump Institute for Molecular Imaging, Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
2Department of Urology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Prostate cancer can benefit from non-invasive and more accurate diagnosis, as well as improved visualization during surgery. Immuno-PET can provide information on extent and location of the disease, while fluorescent image-guided surgery can distinguish cancerous tissue from healthy surrounding...
tissues for clean resection. Prostate Stem Cell Antigen (PSCA) is upregulated in the majority of prostate cancers and metastases and is therefore a promising target for imaging (Knowles *J Nucl Med* 55:429, 2014). Engineered antibody fragments, such as the minibody, exhibit ideal immuno-PET imaging characteristics due to fast blood clearance for high target-to-background images at short imaging times post-injection (Wu *Methods* 65:139, 2014). A dual-labeled minibody probe can reveal the current PSCA-expressing tumor burden by PET, while also identifying margins of malignancy by near-infrared fluorescence.

The humanized anti-PSCA A11 minibody (A11 Mb) was previously affinity matured by yeast scFv display (Lepin Eur. J. Nucl. Med. 37:1529, 2010), then engineered with a C-terminal cys-tag (A11 cMb) that can be site-specifically labeled by thiol-chemistry. 124I-A11 cMb, 124I-Cy5.5-A11 cMb, 89Zr-DFO-A11 cMb, and 89Zr-DFO-A11 cMb-Cy5.5 were used to image 22Rv1 tumors expressing PSCA. For dual-modality imaging, A11 cMb was site-specifically conjugated with Cy5.5-maleimide and radiolabeled with 124I or 89Zr. PET imaging with 124I-Cy5.5-A11 cMb in nude mice bearing subcutaneous PSCA-positive and negative tumors resulted in a positive-to-negative tumor ratio of 13:1 at 22 hours post-injection, comparable to the 8:1 ratio when imaged with 124I-A11 cMb. The PSCA-positive tumors were subsequently visualized by fluorescence *in situ* and *ex vivo* (see Figure 2).

In order to radiolabel with 89Zr, a metal chelator desferrioxamine (DFO) was conjugated to A11 cMb by maleimide chemistry, or SCN-DFO was labeled to random lysines. 89Zr-DFO-A11 cMb demonstrated specific tumor targeting in subcutaneous PSCA-positive tumors. In an orthotopic model, imaging with 89Zr-DFO-A11 cMb-Cy5.5 resulted in a 3:1 tumor-to-blood ratio, and fluorescence clearly distinguished prostate tumor from adjacent tissues. In conclusion, the novel A11 cMb has been successfully used for visualization of tumor burden by immuno-PET and fluorescence imaging, which has the potential for clinical translation.
In Aim 2, we have complete large parts of Task 1, developing relevant models of prostate cancer metastasis to bone and soft tissues through the use of intra-cardiac injections of 22RV1-PSCA and LNCaP-C42b-PSCA cell lines (Figure 3). Imaging has been done with both singly and dually labeled cys-minibody fragments (not shown).

In Aim 3, we have reported on the development of an A2 cys-diabody fluorescently labeled with Cy5 and its ability both to visualize prostate tumors in vivo and to reduce positive margin rates in prostate cancer models. (Sonn GA, Behesnelian A, Zhang Z, Lepin EJ, Bentolila LA, Lawrence DJP, Zettlitz KA, Wu AM, Reiter RE; Fluorescent Image-Guided Surgery with an Anti-Prostate Stem Cell Antigen (PSCA) Diabody Enables Targeted Resection of Prostate Cancer Xenografts in Real-Time. Clinical Cancer Res., 2016). We have further gone ahead to show that intra-operative imaging can reduce recurrence rates of prostate cancer in mice whose tumors were resected with combined white light and fluorescent imaging compared to mice resected with white light alone. We have also optimized NIR labeled cys-diabody and cys-minibody using IR-800 and ICG, which are compatible with operative instrumentation for detection of fluorescence, including the da Vinci robotic system. Similar imaging results were obtained with NIR dyes as with Cy5. We are currently focused on dual PET and fluorescent probes as described above.

What opportunities for training and professional development has the project provided? Nothing to report.

How were results disseminated to communities of interest? Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals? During the next year we will be able to transition our lead dually-labeled PET/optical probes to more biologically relevant models of prostate cancer including bone and lymph node metastasis models as well as genetically engineered mouse models. We will also begin to apply these in intraoperative models of prostate cancer. Finally, we will focus on the dual F-18/optical probes (which will be based on the
smaller A2 cys-diabody because its in vivo kinetics are better matched to the physical half-life of F-18), in collaboration with Dr. Anna Wu’s laboratory.

4. IMPACT What was the impact on the development of the principle discipline of the project? Nothing to report. 

What was the impact on other disciplines? Nothing to report. 

What was the impact on technology transfer? Nothing to report. 

What was the impact on society beyond science and technology? Nothing to report. After only one year of funded research it is too early for our findings to have significant impact.

5. CHANGES/PROBLEMS

Nothing to report. There are no significant changes to the objectives, scope, and approaches of the project.

6. PRODUCTS

Publications, conference papers, and presentations

Abstracts


7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Robert E Reiter
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Dr. Reiter oversaw all aspects of work performed and accomplished to-date.

Funding Support:

Names: Geoffrey Sonn and Andrew Behesnelian
Project Role: Urology Fellow and Residents
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Drs. Sonn and Behesnelain performed the in vivo optical surgical experiments

Funding Support:

Name: Evelyn Kono
Project Role: Senior Research Assistant
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Ms. Kono developed and performed the metastatic models and imaging of said.

Funding Support:

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

The following funding has been renewed and/or is active:

Renewal funding: R01CA174294 Multifunctional immunoPET tracers for pancreatic and prostate cancer (Wu, Reiter, Multi-PIs); time commitment of 1.8 calendar months; renewal project period of 8/1/2016 - 7/31/2021

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

8. SPECIAL REPORTING REQUIREMENTS

Collaborative awards This report covers the activities of the PI Robert E Reiter and Partnering PI Anna Wu.

9. APPENDICES None