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14. ABSTRACT
The humanized antibody to prostate stem cell antigen (PSCA), a cell surface marker on prostate cancer cells, combined with PET scanning was developed to image prostate tumor. The antibody was successful in preclinical model, although humanization caused a loss of affinity relative to the parental monoclonal antibody. To improve the imaging agent, multiple versions of humanized PSCA antibody fragments were created and subsequently tested for their abilities to image in vivo using micro-PET, with the goal to eventually detect distant metastases. Both the engineered minibodies and diabodies were able to successfully detect tumor, and the affinity that was decreased upon humanization was improved. Experiments are ongoing to characterize the affinity matured variant of the minibodies, therefore a lead agent has not been chosen, but this is expected to happen in the next year. On the other hand, a human PSCA knockin mouse model was generated, verified and maintained. This genetically engineered murine line is being expanded and will be used to cross to a known model of mouse prostate cancer in the next year.

15. SUBJECT TERMS
Prostate cancer, prostate stem cell antigen, preclinical mouse model

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INTRODUCTION

The primary imaging modalities for staging of prostate cancer are ultrasound, CT, MRI and MR spectroscopy, bone scan, and PET. Of these methods, bone scan has been a mainstay of prostate cancer staging for many years for detection of bone metastases. However, recent clinical studies using a prostate-specific radiolabeled antibody have reported better detection of bone lesions that were not found by bone scan [1, 2]. Such method, while promising, is still far from being useful due to reports of variable sensitivities and specificities [3, 4]. In addition, molecular targeted therapy of recurrent and metastatic prostate cancer requires the ability to classify tumors at the molecular level without invasive tissue sampling. These can include use of serum markers or markers on circulating cells. One attractive approach is noninvasive molecular imaging, which can yield information on tumor localization, phenotyping, and response to therapy. To this end, we have generated humanized antibody to prostate stem cell antigen (PSCA), a cell surface marker on prostate cancer cells [5, 6], and combined PET scanning to detect binding of this radiolabeled antibody to prostate tumor [7]. While the antibody was able to target tumor in preclinical model *in vivo*, humanization resulted in loss of apparent affinity relative to the parental monoclonal antibody. To further improve pharmacokinetics of the antibody, we proposed to engineer PSCA antibody fragments and test them in preclinical models not only for tumor localization, but also to eventually detect distant metastases.

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Specific Aim 1. Development and testing of engineered PSCA antibody fragments in controlled biological model systems.

Aim 1A. Generation of PSCA-specific antibody fragments (year 1).
Aim 1B. Performance characteristics of anti-PSCA engineered antibody in subcutaneous xenograft models (year 1-2).

Following this aim, we engineered minibodies that recognize PSCA based on the intact humanized PSCA antibody. One candidate, refered to as 2B3 minibody, was expressed successfully to yield about 20 mg/L in culture. Functional characterization of the purified minibody showed specific binding to LNCaP-PSCA cells with relative affinity of 46 nM. When radiolabeled with I-124, and evaluated by PET, the 2B3 minibody localized well to PSCA-expressing LAPC9 xenografts in vivo with excellent contrast at 21 hr after administration. The minibody also showed rapid clearance from non-target tissue and blood. However, reformatting the humanized version of the antibody to the 2B3 minibody resulted in a further 9-fold loss of apparent affinity. Part of this work was submitted and published [8].

To improve affinity and to generate a stable, rapid clearing PSCA-specific antibody fragment, diabodies were engineered from humanized monoclonal antibody. These included four variants with different linker lengths and back-mutations to original murine residues for affinity improvement. The nomenclature for these diabodies are as followed:

- p2B3-Db5: parental 2B3 diabodies with five amino acid linker
- p2B3-Db8: parental 2B3 diabodies with eight amino acid linker
- bm2B3-Db5: back-mutated 2B3 diabodies with five amino acid linker
- bm2B3-Db8: back-mutated 2B3 diabodies with eight amino acid linker.

All diabody variants were evaluated for binding to PSCA by flow cytometry and affinities were determined by surface plasmon resonance. Back-mutation improve the affinity from 5.4 to 1.9 nM. Size exclusion evaluation revealed that diabodies with eight-residue linkers existed as a mixture of dimeric and monomeric species at low concentrations. By shortening the linker from eight to five residues, dimer stability was improved particularly from bm2B3-Db8 to bm2B3-Db5. To test whether the 2B3 diabody would function as an effective PET radiotracer for producing high contrast images at an early time point, both p2B3-Db8 and bm2B3-Db8 were radioiodinated with I-124 and injected into mice bearing LAPC9 human prostate cancer xenografts. Both diabodies were observed to localize in the tumor at 4 h, while at 20 h most of the activity had cleared from the tumor. Highest tumor-to-background contrast ratios and best images were obtained at 12 h. However, biodistribution studies showed that the highest tumor-to-blood ratio was at 8h. The tumor uptake of bm2B3-Db8 was higher than that of the p2B3-Db8 at all time points, and at 20 h it demonstrated improved tumor retention over p2B3-Db8. Nevertheless, bm2B3-Db8 did not improve tumor targeting or imaging compared with p2B3-Db8 at 12 h. Part of this work was submitted and published [9].

Currently, we continued to evaluate affinity matured minibodies for their imaging capabilities using PET.

Aim 1C. Detection of metastatic disease in animal models (year 2): in progress.

Specific Aim 2. Preclinical assessment of PSCA antibody fragments to image transgenic models of prostate cancer.

Aim 2A. Generation of human PSCA knock-in model for evaluation of PSCA antibody fragments (year 1).
A knock-in mouse model was successfully generated, in which the human (h) PSCA cDNA substituted for the murine PSCA gene. The murine PSCA gene was targeted by homologous recombination within the murine embryonic stem cells 129sv (ES). Several positive clones were identified, three were confirmed by Southern blot. One positive clone was expanded and microinjected into donor blastocysts (C57Bl/6), which were implanted in ICR foster mice. The steps involved in creating this model were summarized below (figure 1). Three hPSCA knockin founders were genotyped to confirm presence of hPSCA, but subsequent breeding to expand the line proved difficult. Microinjection was repeated with a different ES clone to generate new sets of founder mice. We were able to breed these new lines pass F1 generation and confirmed germline transmission using a PCR genotyping assay (figure 2).

Currently we have 4 robust transgenic lines, and are screening their organs to determine specific distribution of hPSCA. One optimal line (with higher expression in the prostate) will be chosen to test the imaging agent (engineered PSCA minibodies). Once the optimal line is chosen, we will begin breeding of these mice to obtain homozygotes, which will be used to cross with the conditional PTEN knockout mice.

Aim 2B. Generation of hPSCA knockin X conditional-PTEN knockout (year 2-3): in progress.

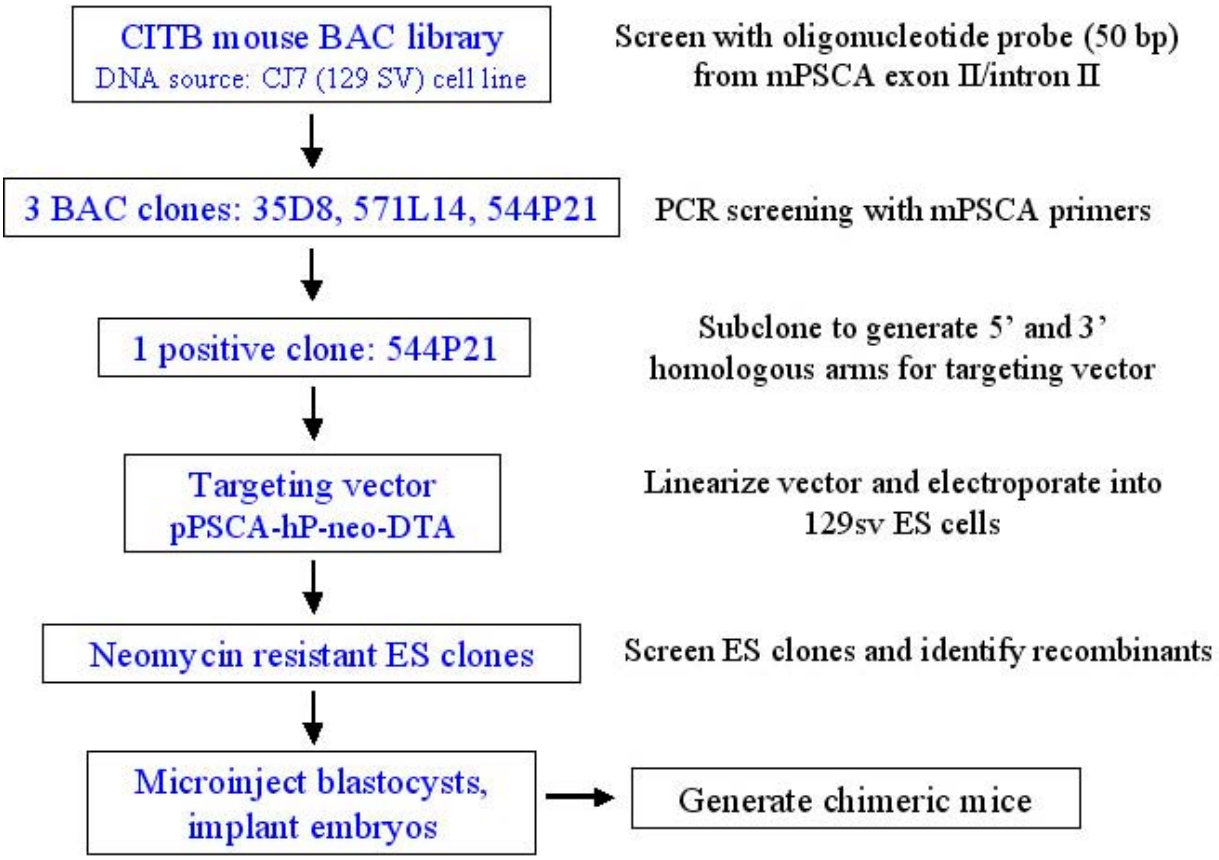

Figure 1. Schematic representation of generation of the hPSCA knockin murine model.

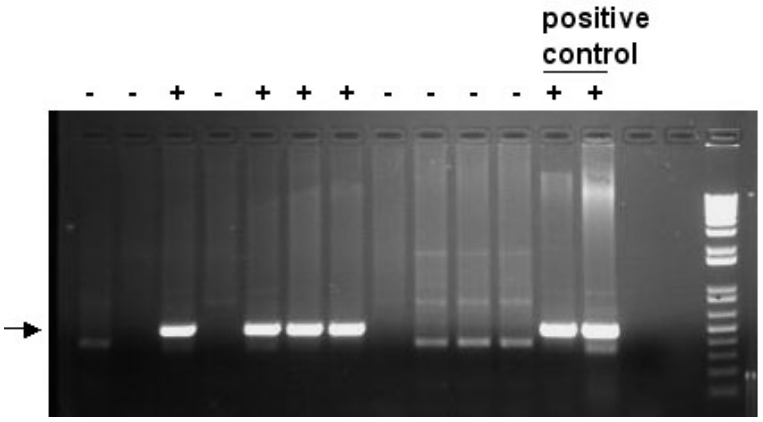

Figure 2. PCR (polymerase chain reaction) genotyping of hPSCA knockin F1 mice.

KEY RESEARCH ACCOMPLISHMENTS

- Successfully generated minibodies and diabodies against PSCA
- Confirmed that back mutation improved diabodies affinity
- Able to test the minibodies and diabodies in vivo imaging in prostate cancer xenograft model: successful imaging of tumor.
- Successfully generated and propagated a hPSCA knockin mouse model.

REPORTABLE OUTCOMES

- Manuscripts published that were supported in part by this grants:
1/ Leyton JV, Olafsen T, Lepin EJ, Hahm S, Bauer KB, Reiter RE, Wu AM. Humanized radioiodinated minibody for imaging of prostate stem cell antigen-expressing tumors. Clin Cancer Res. 2008 Nov 15;14(22):7488-96.

2/ Leyton JV, Olafsen T, Sherman MA, Bauer KB, Aghajanian P, Reiter RE, Wu AM. Engineered humanized diabodies for microPET imaging of prostate stem cell antigen-expressing tumors. Protein Eng Des Sel. 2009 Mar;22(3):209-16.

- Generation of hPSCA knockin animal model.

CONCLUSION

During the past year, we were able to create multiple versions of humanized PSCA antibody fragments and subsequently tested their abilities to image in vivo using micro-PET. Both the engineered minibodies and diabodies were able to detect tumor, and we were able to improve the affinity that was decreased upon humanization. However, we are still carrying out experiments to characterize the affinity matured variant of the minibodies. Therefore we have not narrowed down a lead agent, but expect this would happen in the next year. In parallel, we were able to create, verify and maintain a human PSCA knockin mouse model, which will be used to cross to known model of mouse prostate cancer in the next year.

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