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14. ABSTRACT The goals of the proposed studies are to: i) use imaging methods to non-invasively assess the temporal relationship between castration resistant prostate cancer (CRPC) growth, androgen receptor (AR) levels, angiogenesis, hypoxia, and translocator protein (TSPO) levels and ii) use imaging to temporally direct pathological examination of tissue in order to enhance the elucidation of mechanistic aspects of CRPC progression, specifically the involvement of HIF-1alpha and NF-kappaB, two pathways that increase AR activity during progression to CRPC. During this year of the award focused on characterizing molecular imaging agents in the Pten / p53 double null mutant mouse model. Towards that end, we have successfully acquired anatomic MRI and PET data in orthotopic tumors within the Pten/p53 mouse model, to assess tumor volume, track growth and tumor angiogenesis. We have further characterized the use of FMISO and TSPO imaging to evaluate tumor hypoxia and translocator protein expression. The characterization of these imaging features has found the exciting result that the uptake of a TSPO imaging agent developed in-house shows marked and very specific uptake in all the tumors we observed with MRI.

15. SUBJECT TERMS
castration resistant prostate cancer, MRI, PET, FDHT, image optimization

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Table of Contents

Introduction

In its advanced stages, prostate cancer (PCa) becomes clinically difficult to restrain due to failure of therapy and the development of castration resistant prostate cancer (CRPC). Thus, there is a compelling need to investigate the mechanisms leading to CRPC in order to develop more effective treatment strategies. The most common approach to biologically assess disease progression in mouse models of PCa is through pathological examination, which requires the sacrifice of mice at multiple arbitrary time points and, consequently, is unsuitable for the temporal characterization of physiological, cellular and molecular events leading to CRPC growth in a given animal. In recent years, however, there have been dramatic increases in the range and quality of information available from non-invasive imaging methods so that many potentially valuable imaging metrics are now available to quantitatively measure tumor growth, assess tumor status, and predict treatment response. To this end, our study aims to evaluate emerging, clinically-viable imaging metrics in an appropriate PCa animal model to serially assess tumor progression and establish which method (or combination of methods) is most accurate at predicting castration induced tumor regression and the subsequent recurrence of the castration resistant tumors. In particular, we proposed to non-invasively assess the temporal relationship between CRPC growth, androgen receptor (AR) levels, angiogenesis, hypoxia, cellular proliferation and apoptosis using Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET). Such studies could provide the scientific basis for the acceleration of these emerging imaging methods into clinical care and could have a direct impact on prostate cancer detection, staging and treatment monitoring. Additionally, we proposed to use multi-parametric imaging to temporally direct pathological examination of tissue in order to elucidate mechanistic aspects of CRPC progression, specifically the involvement of hypoxia (HIF-1α) and NF-κB, two essential pathways that increase AR activity during progression to CRPC. The proposed studies are being carried out in the genetically engineered *Pten/p53* conditional mouse model (Ptenpc-/-; *Trp53pc-/-* double-null mutants). Our preliminary studies revealed that prostate tumors in these mutant mice are initially sensitive to castration, as evidenced by tumor regression, but this is followed by tumor recurrence that is ultimately lethal. The regression response to castration and subsequent CRPC growth in these mice clinically recapitulate the disease progression observed in human prostate cancer undergoing androgen-ablation therapy (AAT). Therefore, this authentic mouse model provides a valuable and unique tool to study CRPC progression and dysregulated pathways that could be used as targets of novel therapeutic strategies. Our hypothesis is that multiparametric imaging of vascular, cellular and molecular events will identify stages that predict CRPC progression.

Body

The overall goal of the project in Year 3 was to characterize and compare translocator protein expression (TSPO) and hypoxia (18F-FMISO) imaging of prostate cancer in Pten/p53 mice.

Progress: We aimed to characterize the uptake of a PET tracer targeting translocator protein expression (TSPO), using 18F-VUIIS1008 (a probe developed in-house), and hypoxia, using 18F-Flouromisonidazole (FMISO) in Pten/p53 conditional mice. In the same animals we acquired MRI, TSPO PET and FMISO PET over multiple time points.

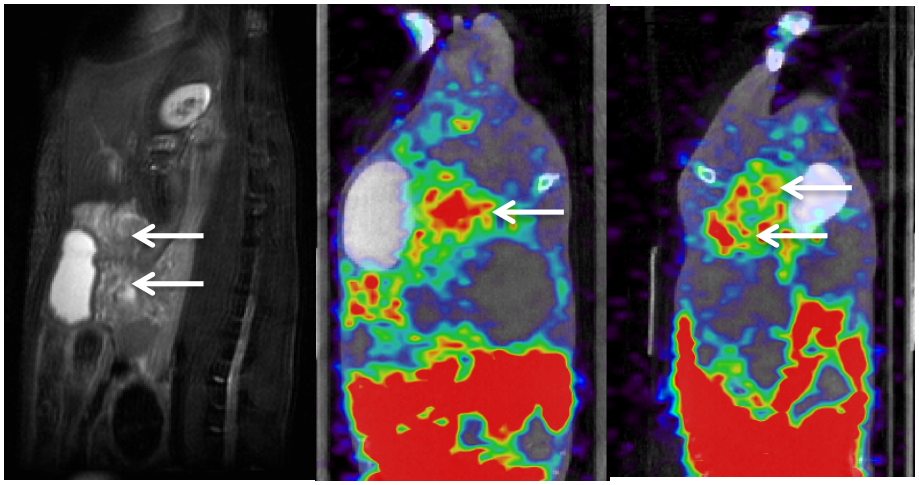

Figure 1: Representative MRI (left), and TSPO PET images (coronal slice center, transverse slice right) in a Pten/p53 mouse model.

Interestingly, unlike our characterization of the androgen receptor probe (FDHT), which we used in prior years, the uptake of TSPO, pre-castration, was highly localized within the prostate tumors and, therefore, exhibited very high tumor to muscle ratios (>4 in most tumors). **Figure 1** shows representative MRI and TSPO PET images. Note the presence of tumor(s) near the bladder in both the MRI and PET images. **Figure 2** highlights the dynamic uptake of TSPO as compared to muscle. Across 60 minutes the %ID/cc continues to increase which is indicative of specific uptake and retention of the tracer. Another feature of this agent that makes it very attractive for prostate cancer imaging is that it shows no excretion via the bladder and minimal uptake in the tissue surrounding the prostate. The only other marked uptake was in liver, which is also apparent in Figure 4. **Figure 3** compares the uptake of VUIIS1008 in tumor and muscle. The radiotracer concentration in tumor was 7.1 ± 1.6 %ID/cc, significantly higher than that of muscle ($p<0.5$) were muscle radiotracer concentration was 0.7 ± 0.2 %ID/cc. Positive staining for TSPO was observed in the stained sections as shown in **Figure 4**. The high and specific uptake of VUIIS1008 in PCa is one of the most compelling achievement of the studies in this project and provide the justification and motivation to evaluate TSPO targeted radiotracers in humans.

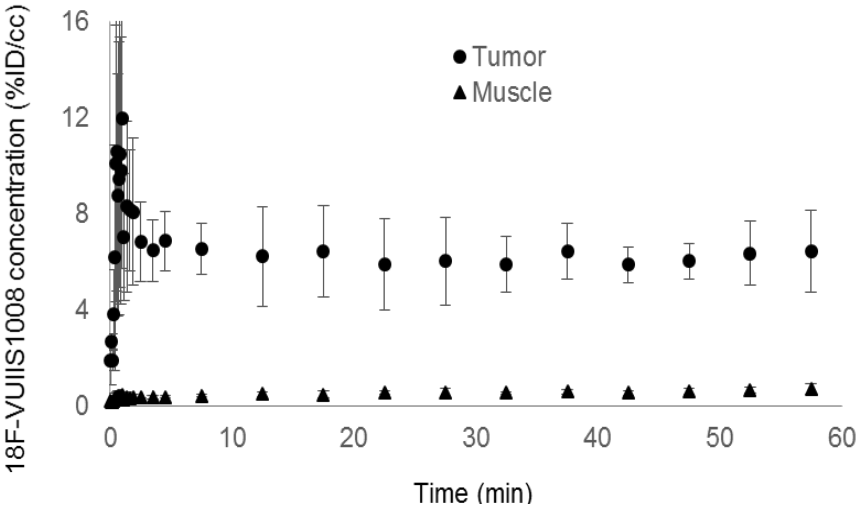

Figure 2: Average time-activity curves (TACs) of mice (n = 9) injected with ^{18}F-VUIIS1008 and imaged in a microPET for 60 min. The error bars are the standard deviation in curves.

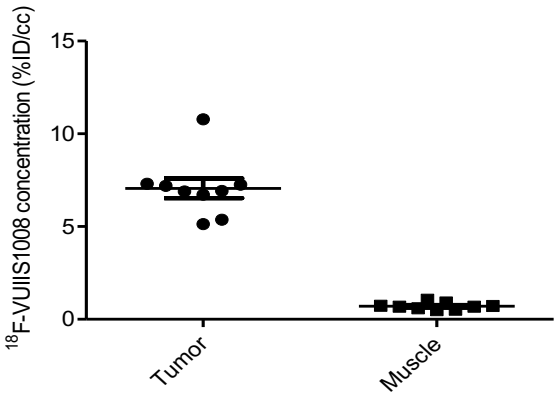

Figure 3: Tumor versus muscle uptake of ^{18}F-VUIIS1008 in mice bearing prostrate tumors.

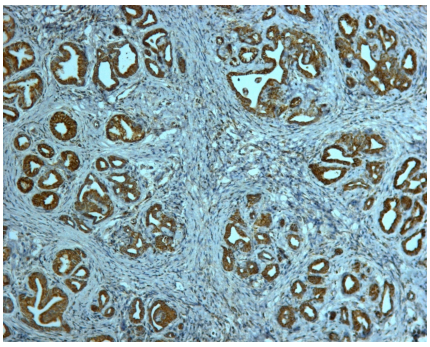

Figure 4: Confirmation of TSPO expression (brown) in a PTEN/p53 mouse model exhibiting high 18F-VUIIS1008 uptake.

Personnel receiving pay from this research effort

C. Chad Quarles, Department of Radiology, Vanderbilt University (10% effort)
Dan Ayers, Department of Biostatistics, Vanderbilt University (5% effort)
Zhenbang Chen, Department of Biochemistry and Cancer, Meharry Medical College
Wenfu Lu, Department of Biochemistry and Cancer, Meharry Medical College

Key Research Accomplishments

- The use of high-resolution anatomic and contrast enhanced MRI methods to track tumor growth in Pten/p53 mouse models
- The first use and characterization of FMISO and TSPO PET compounds in the Pten/p53 mouse model
- This is the first study to demonstrate that TSPO uptake is highly localized in prostate cancer and, accordingly, could improve our ability to detect and track prostate cancer in humans as compared to conventional imaging methods.

Reportable Outcomes

This year was primarily focused on acquiring the data needed to characterize the TSPO imaging agents and since these studies just ongoing we have nothing to report. We are currently preparing a manuscript describing this first use of this agent in PCa. This manuscript, entitled, “Translocator protein PET imaging in a preclinical prostate cancer model” will be submitted to the Molecular Imaging and Biology journal.

Conclusions

In Year 3 we have finished the systematic characterization of the TSPO imaging agent (VUIIS-1008) which holds significant promise for PCa imaging due to his marked uptake as compared to the surrounding tissue. Unlike the other molecular imaging probes we have evaluated in this proposal this agent shows uptake in all the tumors discernable by MRI and was consistently observed across mice. These efforts provide the scientific basis for the evaluation of VUIIS-1008 in patients suffering from PCa, where they could have a direct impact on prostate cancer detection, staging and treatment monitoring.