Award Number:  **W81XWH-11-1-0272**

**TITLE:**  Evaluating the Efficacy of ERG-Targeted Therapy in Vivo for Prostate Tumors

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**CONTRACTING ORGANIZATION:**  Johns Hopkins University
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**REPORT DATE:**  April 2015

**TYPE OF REPORT:**  Annual Summary

**PREPARED FOR:**  U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland  21702-5012

**DISTRIBUTION STATEMENT:**  Approved for Public Release;
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Evaluating the Efficacy of ERG-Targeted Therapy in Vivo for Prostate Tumors

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The proposal centers on developing the principal investigator (PI) into an independent prostate cancer physician-scientist, using as a vehicle this DoD award with specific research aims to examine the ERG oncoprotein as a target for prostate cancer therapy by using novel transgenic mice. As many as 50% of prostate cancers possess a chromosomal translocation involving the \textit{ERG} oncogene. I hypothesized that ERG can serve as an effective molecular therapeutic target for prostate tumors using novel prostate tumor mouse models. During this fourth year of support we have not been able to adhere to our “Statement of Work” – for Task#2 or Task#3. We were successful at completing Task#1, but characterization of ERG expression from our prostate mouse model did not demonstrate any detectable prostate specific ERG expression at the protein level. Data from another project using the \textit{ARR2PB-tTA} line lead us to conclude that the level of expression was insufficient for \textit{in vivo} experimentation. To remedy this issue, we re-started Task #1 last year with the new prostate specific \textit{TET} driver mouse, \textit{Hoxb13-rtTA}. We have spent this last year reinitiating our studies on the ability of ERG to collaborate with \textit{AKT1} with these new mice, \textit{Hoxb13-rtTA/ERG}. We are in the process of breeding more mice and processing samples for analysis. Concurrently during this award period and made possible by this DoD award, the PI has made significant strides in promoting his career as an independently funded prostate cancer physician-scientist with national recognition.

ERG, prostate cancer, inducible transgenic mouse model
## Table of Contents

1. INTRODUCTION............................................................................................................................... 1
2. KEYWORDS........................................................................................................................................... 2
3. OVERALL PROJECT SUMMARY.......................................................................................................... 2
4. KEY RESEARCH ACCOMPLISHMENTS.............................................................................................. 5
5. CONCLUSION...................................................................................................................................... 5
6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS.................................................................... 6
7. INVENTIONS, PATENTS AND LICENSES............................................................................................ 10
8. REPORTABLE OUTCOMES.................................................................................................................. 10
9. OTHER ACHIEVEMENTS................................................................................................................... 10
10. REFERENCES.................................................................................................................................... 13
11. APPENDIX....................................................................................................................................... 14
Evaluating the efficacy of ERG targeted therapy in vivo for prostate tumors
PI – Phuoc T. Tran, MD, PhD

1. INTRODUCTION:
The proposal centers on developing the principal investigator (PI) into an independent prostate cancer physician-scientist, using as a vehicle this DoD award with specific research Aims to examine the ERG oncoprotein as a target for prostate cancer therapy by using novel transgenic mice. Prostate cancer is the most common cancer diagnosed in men in the United States. It has been estimated that greater than 200,000 new cases of prostate cancer were diagnosed in the United States in 2012 and prostate cancer was responsible for ~30,000 deaths or the second most common cause of cancer deaths in men (1). Recent efforts to classify distinct molecular subtypes of prostate cancer have led to the novel findings that greater than 50% of prostate cancers possess a chromosomal translocation involving the ETS oncogene family of transcription factors (2, 3). These ETS translocations result in dysregulated overexpression of the ETS oncogene in prostate cancer cells. The most common ETS family member involved in these translocation events is the v-ets erythroblastosis virus E26 oncogene homolog (ERG). Most molecular targeted therapies in other cancers are notable for their lack of serious side-effects and amazing tolerability. I hypothesized that ERG, the most common ETS oncogene found to be mutated in prostate cancer can serve as an effective molecular therapeutic target for prostate tumors. I planned to show this with novel autochthonous prostate tumor mouse models. I also hypothesized that ERG facilitates tumorigenesis alone or in the context of activated AKT1 by dysregulating proliferation, apoptosis and/or senescence programs in vivo. Demonstrating whether prostate tumors in mouse models are dependent for ERG for tumor survival would be the first proof of principle demonstration of molecularly targeted therapy for spontaneously arising prostate tumors in living animals. Ultimately, this mentored award has the goal of protecting the research time of the PI to allow development of his research program so that he may become a future leader in prostate cancer research.

The original specific aims are below:

Specific Aim#1 - Generate and characterize an inducible ERG prostate specific mouse model.
**Rationale:** I have created a novel prostate TET system mouse model and am interested in the effects of ERG expression alone and in combination with AKT1 in the prostate.
**Study Design:** I will validate inducible expression of both ERG and Luc in vivo using real time-RT-PCR (qPCR), BLI of whole living animals and by organ Western analysis in bi-transgenic ARR2PB-tTA/ ERG-tetO-Luc (AE) mice.

Specific Aim#2 – Determine if ERG cooperates with AKT1 for prostate tumorigenesis.
**Rationale:** ERG overexpression in vitro suggests that ERG may facilitate tumorigenesis, but ERG transgenic mouse models vary in the severity of their tumor phenotypes alone and with AKT1 co-overexpression. The mechanism for ERG prostate phenotypes alone or in combination with AKT1 overexpression in vivo are unknown.
**Study Design:** Generate ARR2PB-tTA/MPAKT1/ ERG-tetO-Luc (AA1E) tri-transgenic mice and compare to single oncogene mice to genetically analyze cooperation in vivo. Investigate using molecular techniques if ERG modulates proliferation, apoptosis and/or senescence programs in vivo.

Specific Aim#3 - Determine if ERG can serve as an effective molecular therapeutic target for prostate tumors in vivo.
**Rationale:** Despite the importance that ERG overexpression is believed to play in prostate tumorigenesis, the therapeutic value of targeting ERG on autochthonous prostate tumors has not been tested in vivo. The mechanism for any autochthonous tumor regression or stasis in vivo upon ERG inactivation is unknown.
**Study Design:** Following development of autochthonous prostate tumors in TET regulated mice I will treat mice with doxycycline to simulate targeted treatment against the ERG oncogene. Investigate using molecular techniques if ERG inactivation modulates proliferation, apoptosis and/or senescence programs in autochthonous prostate tumors in vivo.
2. KEYWORDS:
ERG
Prostate cancer
Inducible transgenic mouse model

3. OVERALL PROJECT SUMMARY:
Progress is listed in relation to each specific task in the “Statement of Work” and highlighted by *italics* for Years 1-3 and **BOLD** font for the past year (Year 4).

Task#1 - Generate and characterize an inducible ERG prostate specific mouse model (months 1-17).
Numbers of mice surviving weaning and for mating: 65
1a. IACUC and other regulatory approval process for animal work (months 1-4).
As reported in our Year 1 Progress Report, we applied for and obtained approval from the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center IACUC for the studies described in our DoD grant award (see Appendix for documentation approval).

1a. Mating mice to characterize (months 4-10).
As reported in our Year 1 Progress Report, the appropriate single transgene ARR2PB-tTA (A) and ERG-tetO-Luc (E) mice were mated to produce cohorts of (AE) bitransgenic mice. There were no issues with producing the required numbers of AE mice.

We are still in the process of generating sufficient numbers of bitransgenic, Hoxb13-rtTA/ERG-tetO-luc (HE) and tritransgenic animals, Hoxb13-rtTA/MPAKT1/ERG-tetO-luc (HA1E) mice to perform Task #1 and Task #2, respectively. We have performed some preliminary characterization of the HE mice using as a reporter, functional expression of luc detected with bioluminescene imaging (BLI) and molecular characterization with qPCR and Western for ERG (data not shown; see Table 1B). We do not see robust BLI signal from the HE mice, nor have we observed high level of ERG expression at the mRNA or protein levels (data not shown). This is in contrast to what we observed with a very similar line Twist1-tetO-luc crossed to the prostate specific TET driver Hoxb13-rtTA (4)(see Figure 1 Appendix). Similarly, we have shown that ERG-tetO-luc can express luc from other tissue specific drivers such as those that drive expression ubiquitously (see Figure 2 Appendix) or to the liver (data not shown). Thus, there is the formal possibility that the HE combination is not compatible, but we are still at a preliminary stage.

1b. Collecting tissues from AE mice to characterize ERG expression (months 8-14). AE mice will be weaned and placed on water without doxycycline and 5 males for each of the following age time points: 4, 8, 12 and 24 weeks (n=25 mice total, 5 additional for incidentals), will be interrogated using the assays mentioned below in 1d.
As reported in our Year 1 Progress Report, the appropriate numbers of AE bitransgenic mice (n=25) have been placed on drinking water without doxycycline to activate the ERG transgene.
Pending creation of more HE mice, but we have some mice in the >4 week time point with and without doxycycline (Table 1B).

1c. Collecting tissues from AE mice turned OFF to characterize inducible ERG expression (months 8-14). 12 week old males will be followed for the OFF time points: 1, 2 and 4 weeks (n=20 mice total, 5 additional for incidentals) and tissues extracted for interrogation using the assays mentioned below in 1d.
As reported in our Year 1 Progress Report, the appropriate numbers of AE bitransgenic mice have been placed on regular water (n=20) for 4-6 weeks following weaning to activate the ERG transgene followed by changing to doxycycline drinking water (0.2 mg/ml) changed weekly to inactivate the ERG transgene.

In process of generating sufficient numbers of HE mice.
1d. Performing experiments on tissues from mice (months 14-17). Tissues from 1b and 1c above will be harvested for histology and flash frozen for molecular studies: prostate lobes, other genitourinary (GU) organs, lungs, heart, liver and spleen. These specimens will then be processed for H&E histology and immunohistochemistry (IHC) performed using anti-Myc, anti-FLAG and anti-luciferase antibodies to confirm prostate luminal cell epithelia expression. Whole lobe and organ Western blotting using the same antibodies will also be performed and transcription of ERG confirmed with specimens using qPCR. See Table 1 and 2 below for summary of results. We were able to harvest as above for all the “ON” time points at least 5 mice: 4, 8, 12 and 24 weeks. Similarly, for the “OFF” time points we have been able to collect tissues from ≥ 5 mice from the 1, 2 and 4 week time points.

In process.

We have performed analysis as summarized below in Table 1A & 2A. The AE mice from the “ON” time points collected have had no abnormalities on gross or H&E examination of their prostates. The other organs in these mice (lungs, heart, liver and spleen) were also normal on necropsy. Similarly, the AE mice from the “ON” and “OFF” time course displayed no pathology on gross or histologic exam of the H&E slides. We have attempted IHC and westerns for protein expression of ERG that is tagged by Myc and FLAG epitope tags, but have not been able to see expression using either approach. We also attempted on a limited scale luc IHC and ERG qPCR with these samples which were similarly negative.

In process but we have performed some preliminary analysis as summarized below in Table 1B & 2B.

1e. Analyzing results of experiments on tissues from mice (months 14-17).
See Table 1 and Table 2 for summary of results and “Conclusions” below for explanation of results.

### Table 1A – Summary of Task #1b to date.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>4 wks On DOX</th>
<th>8 wks On DOX</th>
<th>12 wks On DOX</th>
<th>24 wks On DOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>6 mice</td>
<td>7 mice</td>
<td>5 mice</td>
<td>Pending</td>
</tr>
<tr>
<td>Gross</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
</tr>
<tr>
<td>Histologic</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
</tr>
<tr>
<td>Myc IHC</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>Negative expression</td>
</tr>
<tr>
<td>FLAG IHC</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>Negative expression</td>
</tr>
<tr>
<td>luc IHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Negative expression</td>
</tr>
<tr>
<td>FLAG Western</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>ND</td>
<td>Negative expression</td>
</tr>
<tr>
<td>ERG qPCR</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Negative expression</td>
</tr>
</tbody>
</table>

### Table 1B – Summary of Task #1b to date.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>4 wks On DOX</th>
<th>8 wks On DOX</th>
<th>12 wks On DOX</th>
<th>24 wks On DOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>4 mice</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
</tr>
<tr>
<td>Gross</td>
<td>WNL</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
</tr>
<tr>
<td>Histologic</td>
<td>WNL</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
</tr>
<tr>
<td>Myc IHC</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
</tr>
<tr>
<td>FLAG IHC</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
<td>Pending</td>
</tr>
</tbody>
</table>
Many of the steps/tasks below are dependent on the steps above and have not been initiated.
Task#2 - Determine if ERG cooperates with AKT1 for prostate tumorigenesis (months 14-34).
Numbers of mice surviving weaning and for mating: 150
2a. Mating mice for cooperation experiments (months 14-20).
Although, we have not completed all the characterization in Task #1 for HE mice as described above, we have initiated the mating required to produce Hoxb13-rtTA/MPAKT1/ERG-tetO-Luc (HA1E) mice.

2b. Collecting tissues from cooperation experiments (months 18-30).
2c. Performing experiments on tissues from mice (months 20-32). Tissues from 2b above will be harvested for histology and flash frozen for molecular studies: prostate lobes, other GU organs, lungs, heart, liver and spleen. These specimens will then be processed for H&E histology and IHC performed using anti-Myc, anti-FLAG and anti-luciferase antibodies. Whole lobe and organ Western blotting using the same antibodies will also be performed and transcription of ERG confirmed with specimens using qPCR. IHC for cleaved caspase 3 (CC3) and Ki-67. Senescence markers such as p15, p16, p21 and p27 will be analyzed by IHC and qPCR. In addition, I will perform senescence associated beta-galactosidase (SA-β-gal) staining.
2d. Analyzing results of experiments on tissues from mice (months 22-34).

Each of the steps/tasks below are dependent on the steps above and have not been initiated.

Task#3 - Determine if ERG can serve as an effective molecular therapeutic target for prostate tumors in vivo (months 34-60)
Numbers of mice surviving weaning and for mating: 120
3a. Mating mice for therapeutic experiments (months 34-40).
3b. Collecting tissues from therapeutic experiments mice ON 6-12 months and then OFF 1-6 months (months 40-56).
3c. Performing experiments on tissues from mice (months 42-58). Tissues from 3b above will be harvested for histology and flash frozen for molecular studies: prostate lobes, other GU organs, lungs, heart, liver and spleen. These specimens will then be processed for H&E histology and IHC performed for Myc, FLAG, luciferase, CC3, Ki-67, p15, p16, p21 and p27. Whole lobe and organ Western blotting using the same antibodies will also be performed and transcription of ERG confirmed with specimens using qPCR. In addition, I will perform SA-β-gal staining.
3d. Analyzing results of experiments on tissues from mice (months 44-60).

4. KEY RESEARCH ACCOMPLISHMENTS:

- Confirmation that our ARR2Pb-tTA mouse line is not robust enough to drive expression of tetO-regulated genes in the mouse prostate.
- Development of novel bitransgenic, Hoxb13-rtTA/ERG-tetO-Luc (HE) and tritransgenic animals, Hoxb13-rtTA/MPAKT1/ERG-tetO-Luc (HA1E) using the more robust prostate specific driver Hoxb13-rtTA.
- Initial characterization of the HE mice and continued breeding to produce more HE for Task #1 and HA1E mice for Task #2.

5. CONCLUSION:
During this fourth year of support we have not been able to adhere to the timeline of our “Statement of Work” - Task#2 - Determine if ERG cooperates with AKT1 for prostate tumorigenesis (months 14-34) or Task#3 - Determine if ERG can serve as an effective molecular therapeutic target for prostate tumors in vivo (months 34-60). We were previously successful at completing the tasks for Task#1 - Generate and characterize an inducible ERG prostate specific mouse model (months 1-17), but this characterization of ERG expression from our old prostate inducible mouse model, ARR2PB-tTA, did not demonstrate any detectable prostate specific ERG expression at the protein level using Western or IHC (see Tables 1A & 2A above).
However, characterization of the ERG founder lines indicated that expression was feasible using a different promoter element driving a similar tTA gene in the liver (data not shown) and another rtTA mouse line, CMV-rtTA (CMV), where inducible expression is ubiquitous (Figure 2 Appendix).

We had in our Year 2 progress report concluded that the lack of a prostate phenotype despite prostate epithelium specific expression of other tetO reporter lines was due to the level of expression from the ARR2PB-tTA line was too low and perhaps insufficient for the in vivo experiments described in our proposal.

In Year 3 we attempted to remedy this issue with low prostate specific expression and proposed to restart Task #1 of the project with the new prostate specific TET driver mouse, Hoxb13-rtTA (H) (4), in collaboration with Dr. Charles Bieberich. The Hoxb13-rtTA line allows for much more robust expression of tetO target genes than our ARR2PB-tTA line (see Figure 1 Appendix). The breeding between our tetO-ERG mice and Dr. Bieberich’s Hoxb13-rtTA mice has been problematic, but we overcame some of these issues and have initial preliminary data with Hoxb13-rtTA/ tetO-ERG (HE) mice (see Table 1B). Unfortunately, we have not seen expression with with BLI, via qPCR for ERG or Western for ERG in HE mice (data not shown). We are currently trouble shooting this current situation. Regardless, we are now in the process of reinitiating our Task #1 studies with these new HE mice (see Tables 1B & 2B above). Thus we are still optimistic that our tetO-ERG lines are capable of inducible prostate specific ERG expression using the TET inducible prostate mouse model, Hoxb13-rtTA (4) and hope to finish Task #1 and part of Task #2 in the upcoming final year of the DoD award.

Finally, the ultimate goal of this DoD Prostate Cancer Physician Research Training Award (PRTA) was to help develop the PI into an independent prostate cancer researcher. Concurrently during this entire award period and made possible by this DoD award, the PI has made significant strides in promoting his career as an independently funded prostate cancer physician-scientist with national recognition.

“So What”

Despite the importance that ERG overexpression is believed to play in prostate tumorigenesis, the therapeutic value of targeting ERG rearrangements has not been tested in vivo. The ability to interrogate using in vivo model systems whether ERG or other oncogenes are good molecular therapeutic targets could provide a huge leap forward for prostate cancer research and treatment of prostate cancer patients. Demonstrating whether prostate tumors in my inducible transgenic mice are dependent for ERG for tumor maintenance would be the first proof of principle demonstration of molecularly targeted therapy for prostate tumors in vivo and we will be able to determine whether molecularly targeted therapy against ERG in the context of activated AKTI1 would be an effective therapy for prostate tumors.

The ultimate goal of this DoD PRTA was to develop the PI into an independent prostate cancer researcher. The PI has made significant strides in promoting his career as an independently funded prostate cancer physician-scientist with national recognition.

6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS:

- During this fourth year of support we have not published any manuscripts, abstracts or presented work directly from the Aims proposed in this DOD PRTA at any venue other than at our own private lab meetings. However, in the spirit of this award protecting the research time of the PI, this has enabled our group to contribute the following reportable outcomes related to prostate cancer research:

  1. Lay Press:

     Nothing to report.

  2. Peer-Reviewed Scientific Journals (Since the beginning of the DoD PRTA):


       + - corresponding author.

* - these authors contributed equally.


* - these authors contributed equally.


* - these authors contributed equally.

** - Cover illustration and Highlighted in *Mol Cancer Res*.


* - these authors contributed equally.


3. Invited Articles (Since the beginning of the DoD PRTA):

4. Abstracts (Year 4 only from a total of 27 since the beginning of the DoD PRTA):
   1. Rajendra P. Gajula, Russell D. Williams, Reem Malek, Katriana Nugent, Amanda J. Walker, Sivarajan T. Chettiar, Hailun Wang, Kekoa Taparra, Jessica Cades, Steven An, Joseph M. Herman, **Phuoc T. Tran**. A TWIST1-MLL-HOTTIP complex regulates *HOXA9* chromatin to facilitate metastasis in prostate cancer. **Poster discussion** for the ASTRO 2014 National Meeting.
   8. Rajendra P. Gajula, Russell D. Williams, Reem Malek, Katriana Nugent, Sivarajan T. Chettiar, Hailun Wang, Kekoa Taparra, Jessica Cades, Joseph M. Herman, **Phuoc T. Tran**. A TWIST1-MLL-WDR5-HOTTIP complex regulates HOXA9 chromatin to facilitate metastasis of prostate cancer. **21st Annual Prostate Cancer Foundation Meeting 2014**.


**SEMINARS/TALKS (Since the beginning of the DoD PRTA):**
1. World Presidents’ Organization Health Network Foundation Program for JHU Men’s Health Day (November 20, 2010). “Prostate Cancer: Prevention, Screening and Treatment Options”.

2. RTOG Semi-annual Meeting - Genitourinary Translational Research Program (January 14, 2011). “MYC as a biomarker to direct statin targeted radiosensitization for definitive treatment of prostate cancer”.

3. George O’Brien Center at Johns Hopkins University Advisory Committee Meeting (January 25, 2011). “**TWIST1** and Embryonic Reawakening in benign prostatic hyperplasia revisited”.


5. JHU, Brady Urology Prostate Cancer Advisory Board Meeting (June 5, 2012). “Using High-Dose Statins to Target MYC-overexpressing Prostate Cancers”.

6. I Congress of Oncology D’Or (Rio de Janeiro, Brazil) – Meeting with Johns Hopkins Experts (July 6, 2013). “Extreme Hypofractionation for Localized Prostate CA: Radiobiologic Rationale & Early Results”.

7. Stanford University Medical Center, Radiation Oncology Visiting Professor (October 28, 2013). “Structure-functions studies of the **TWIST1** oncoprotein in lung and prostate cancer”.


10. JHU, Brady Urology Prostate Cancer Research Day (February 8, 2014). “Phase I Trial of HSP90 inhibition and radiation-androgen deprivation therapy for high-risk, localized and locally advanced prostate cancer”.

11. UC San Diego, Moores Cancer Center (April 18, 2014). “Structure-functions studies of the **TWIST1** oncoprotein in lung and prostate cancer”.


15. JHU, SKCCC Translational Research Conference (February 11, 2015). “Credentialing **TWIST1** as a Therapeutic Target in Lung and Prostate Cancer”.

16. JHU, Brady Urology Prostate Cancer Working Group (February 20, 2015). “Consolidative Local Therapies for Oligometastatic Prostate Cancer”.

Page 9
17. JHU, Radiation Oncology Grand Rounds (February 24, 2015). “Credentialing TWIST1 as a Therapeutic Target in Lung and Prostate Cancer”.
18. 8th Multi-institutional Prostate Cancer SPORE Retreat (March 16, 2015). “Stereotactic Ablative Radiation for Treatment of Oligometastatic Disease”.
19. Georgetown University Medical Center, Biochemistry and Molecular & Cellular Biology (March 31, 2015). “Credentialing TWIST1 as a Therapeutic Target in Lung and Prostate Cancer”.

7. INVENTIONS, PATENTS AND LICENSES:
Nothing to report.

8. REPORTABLE OUTCOMES:
Nothing to report.

9. OTHER ACHIEVEMENTS:

CLINICAL TRIALS (PI only since the beginning of the DoD PRTA):
2. J1153 - Pharmacodynamic Trial of Pre-Prostatectomy Lovastatin on MYC Down-Regulation in Localized Prostate Cancer. Role: PI. Closed early.
5. Phase I Trial of HSP90 Inhibition and Radiation-Androgen Deprivation Therapy for High-Risk Localized and Locally Advanced Prostate Cancer. Role: PI. Under review CRC/IRB.

GRANTS (Since the beginning of the DoD PRTA):
No additional funding was applied for based on this work specifically resulting from the proposed Aims. However, in the spirit of this award protecting the research time of the PI, this has enabled our group to apply for additional funding related to prostate cancer research:

CURRENT:
1. 1U01CA183031-01A1 Pomper/DeWeese (PI) 5/15/2015-3/31/2017 NIH/NCI
“PSMA-Directed PET/MR Imaging and Image-Guided Therapy of Prostate Cancer”
The overall goal is to validate a positron-emitting, PSMA-targeted imaging agent clinically so it may be used to full advantage in supporting existing and emerging therapies for a spectrum of patients suffering from prostate cancer.
Role: Co-I

2. ENZA-13L21 Tran/Antonarakis (PI) 3/2/2015-1/1/2020 Astellas-Medivation Pharma
“SALV-ENZA - Phase II Randomized Placebo-Controlled Double-Blind Study of Salvage Radiation Therapy (SRT) Plus Placebo vs. SRT Plus Enzalutamide in Men with High Risk PSA-Recurrent Prostate Cancer”
Randomized, double-blind, phase II, prospective, multicenter study in male adults with biochemically recurrent prostate cancer following radical prostatectomy.
Role: co-PI
COMPLETED:
3. O’Brien Center Pilot Tran/Schaeffer (PI) 11/1/10-10/31/12
George M. O’Brien Center for Benign Prostate Hyperplasia/LUTS Research
“TWIST1 and Embryonic Reawakening in benign prostatic hyperplasia revisited”
Aim #1 is to establish a link between prostate luminal cell specific Twist1 overexpression and increased prostate stem cells. Aim #2 is to determine if prostate specific Twist1 overexpression in vivo results in autochthonous prostate hyperplasia.
Role: PI

4. PCW Award Tran (PI) 4/1/11-3/31/13
Patrick C. Walsh Prostate Cancer Research Fund
“MYC as a biomarker to direct statin targeted therapy for definitive treatment of prostate cancer”
Aim #1: Pharmacodynamic Phase 0 trial of pre-prostatectomy lovastatin to downregulate MYC in localized prostate cancer.
Role: PI

5. PCW Award Tran (PI) 4/1/13-3/31/14
Patrick C. Walsh Prostate Cancer Research Fund
“Phase I Trial of HSP90 Inhibition and Radiation-Androgen Deprivation Therapy for High-Risk Localized and Locally Advanced Prostate Cancer”
Phase 1 trial of ganetespib and standard of care radiation and long-term androgen deprivation therapy for high-risk and locally advanced prostate cancer.
Role: PI

6. W81XWH-11-1-0336 Schaeffer (PI) 11/1/11-9/29/14
Dept of Defense CDMRP Prostate Cancer Research Program
“RNASEH2A - a Putative “Non-Oncogene Addiction” Gene Target and Marker for Radio-sensitivity in High Risk Prostate Cancer”
Specific Aim 1: Demonstrate the association of RNASEH2A with lethal prostate cancer. Specific Aim 2: Evaluate the ability of RNASEH2A to modulate radio-sensitivity in prostate cancer cell lines and xenograft models. Specific Aim 3: Investigate RNASEH2A as a tissue and biofluid based marker of radio-sensitivity.
Role: Co-I

EMPLOYMENT/OPPORTUNITIES (Since the beginning of the DoD PRTA):
No employment or research opportunities applied for and/or received based on experience/training specifically from the Aims of this award. However, in the spirit of this award protecting the research time of the PI, this has enabled the PI to be awarded the following opportunities related to prostate cancer research or promote his career:

PROFESSIONAL POSITIONS (Since the beginning of the DoD PRTA):
2010-2013 Assistant Professor, Oncology (secondary appt), JHU SOM.
2010- Member, Sidney Kimmel Comprehensive Cancer Center (SKCCC), JHU SOM.
2012- Member, Graduate Program in Cellular and Molecular Medicine (CMM), JHU SOM.
2013 Assistant Professor, Urology (secondary appt), JHU SOM.
2013- Associate Professor, Radiation Oncology, Oncology and Urology, JHU SOM.
2015- Clinical Director, Radiation Oncology and Molecular Radiation Sciences, JHU SOM.

AWARDS & HONORS (Since the beginning of the DoD PRTA):
2012 Association of Residents in Radiation Oncology (ARRO) Educator of the Year.
2012  Top Doctors by Baltimore Magazine.
2012-2016 American Cancer Society Research Scholar.
2013  OHSU SOM Alumni Association Early Career Achievement Award (Inaugural Award).
2013  Alpha Omega Alpha Honor Medical Society Alumni Award, OHSU Chapter.
2013  The Irene and Bernard L. Schwartz Scholar - Patrick C. Walsh Prostate Cancer Research Fund Award.
2013-2015 Sidney Kimmel Translational Scholar Award.
2013-2018 National Cancer Institute (NCI) 1R01CA166348-01A1.
2015-2016 American Society of Clinical Oncology (ASCO) Leadership Development Program.

COMMITTEES & PROFESSIONAL ACTIVITIES (Since the beginning of the DoD PRTA):
2011  Co-Chair of JHU SOM Radiation Oncology and Molecular Radiation Sciences Modulating Radiation Response - Cancer Fundamentals to Therapy Symposium
2011-2013 ASTRO Scientific Program Committee - Biology Subcommittee
2011-2015 SKCCC Oncology Grand Rounds, Co-Organizer
2011- SKCCC Service of Remembrance Steering Committee
2011- SKCCC Educational Committee
2011- JHU SOM Clinical Practice Association – Compliance Committee
2011- JHU SOM Clinical Practice Association – Clinical Documentation Excellence Program
2011- SKCCC Oncology Animal Facility Advisory Committee
2012 DoD PCRP Clinical and Experimental Therapeutics-2, Ad Hoc Reviewer
2012 JHU John G. Rangos, Sr., Award for Creativity in Cancer Discovery, Ad Hoc Reviewer
2012-2013 DoD Prostate Cancer Research Program (PCRP) Pathobiology-1, Scientist Reviewer
2012-2013 NSCOR - Space Radiation Solid Cancer Risks, Panel Progress Reviewer
2012-2013 JHU Patrick C. Walsh Prostate Cancer Research Fund, Scientist Reviewer
2012-2015 ASTRO Radiobiology Practice Exam and Study Guide Committee of the Science Council
2012-2014 Radiation Oncology Institute National Radiation Oncology Registry (NROR) Pilot Committee
2012- RSNA Research and Education (R&E) Foundation - Radiation Oncology Research Study Section
2013 Prostate Cancer UK Pilot Grant, Ad Hoc Reviewer
2013 DoD PCRP Pathobiology-1, Pre-application Reviewer
2013-2015 SKCCC Clinical Research Review Committee
2013-2015 ASTRO Research Grants Evaluation Committee of the Science Council
2014 NSCOR - Space Radiation Solid Cancer Risks and Biological Countermeasures, Panel Reviewer
2014-2015 ASTRO NROR Pilot Sites Working Group Committee
2014-2015 ASTRO Molecular Targeting White Paper Committee
2014- JHU SOM Instructor/Assistant Professor Reappointment Review Committee
2014- SKCCC Johns Hopkins-Allegheny Health Network Cancer Research Fund, Co-Director
2015 RSNA R&E Foundation – Radiation Oncology Research Study Section, Vice Chair
2015 NIH NCI R03/R21 Program Special Emphasis Panel - ZCA1 SRB-C (M1) S, Reviewer
2015 JHU Patrick C. Walsh Prostate Cancer Research Fund, Scientist Reviewer
2015 ASTRO Scientific Program Committee - Biology Subcommittee
2015 Israel Science Foundation, Scientist Reviewer
2016-2018 RSNA R&E Foundation – Radiation Oncology Research Study Section, Chair

CONFERENCE ORGANIZER, SESSION CHAIR (Since the beginning of the DoD PRTA):
2011 JHU SOM Radiation Oncology and Molecular Radiation Sciences Modulating Radiation Response - Cancer Fundamentals to Therapy Symposium, Co-Chair
2011 ASTRO National Meeting, Session HH - Nanoparticles and Viruses in Radiotherapy, Co-Chair
2011 RSNA National Meeting, Radiation Oncology & Radiobiology - Biology, Co-Chair
2011 RSNA National Meeting, BOOST: Genitourinary – Integrated Science & Practice Session, Co-Chair
2012 ASTRO National Meeting, Session HH - Nanoparticles and Viruses in Radiotherapy, Co-Chair
2012-2013 Radiation Research Society (RRS) Annual Meeting Program Committee
2013 RRS National Meeting, Topical Review – Recent Advancements in Production of Genetically Engineered Mice, Chair
2013 RRS National Meeting, Symposium – Immune Modulation and Radiation Strategies - Improving Local and Abscopal Responses, Chair
2013 ASTRO National Meeting, Session F - DNA Damage and Repair: Novel Biological Principles and Targeted Radiosensitization Strategies, Co-Chair
2013 RSNA National Meeting, Radiation Oncology & Radiobiology - Genitourinary, Co-Chair
2013 RSNA National Meeting, BOOST: Genitourinary – Integrated Science & Practice Session, Co-Chair
2014 Prostate Cancer UK 11th Biennial Prostate Cancer Forum – Management: Low-Risk Disease, Co-Chair
2014 RRS National Meeting, Symposium – Radiation Response of Normal Tissue Stem Cells, Chair
2014 ASTRO National Meeting, Session SS X - Biology 3 - Biomarkers and Imaging, Co-Chair
2014 JHU SOM Radiation Oncology and Molecular Radiation Sciences Annual Research Retreat, Co-Organizer

EDITORIAL ACTIVITIES (Since the beginning of the DoD PRTA):
2014- Cancer Research, Associate Editor – Breaking Advances

10. REFERENCES:
11. APPENDIX:

Fig 1. Generation of an inducible luc prostate epithelial specific mouse model. Mice containing a prostate specific TET driver transgene, Hoxb13-rTA was crossed with a reporter mouse luc-tetO-Twist1 line to produce bi-transgenic animals (HT). The presence of doxycycline allows the rTA protein to bind and activate the tetO promoter. Removal of doxycycline triggers a conformational change which prevents tetO binding, activation and inhibits Twist and luc transcription. HT animals express luciferase inducibly in the prostate as shown by bioluminescence imaging (BLI) (ip injection with luciferin substrate and imaged 10 minutes later on a Xenogen Spectrum machine shows a colored bright region in the lower abdomen/high pelvis). Dox – doxycycline was given to animals in the drinking water [2 mg/ml]. Animal 1 has a Hoxb13-rTA genotype and animal 2 is an HT mouse. The smaller panels on the right are animal 2 after necropsy and dissection of the prostate and seminal vesicles. In these right panels prostate inducible and specific luc expression can be seen by BLI.

Fig 2. Generation of an inducible luc-tetO-ERG mouse model. Mice containing a ubiquitous TET driver transgene, CMV-rTA were crossed with our ERG line of interest, luc-tetO-ERG, that has the luc reporter to generate bi-transgenic animals (CMV-E). The presence of doxycycline allows the rTA protein to bind and activate the tetO promoter. Removal of doxycycline triggers a conformational change which prevents tetO binding, activation and inhibits ERG and luc transcription. CMV-E animals express luciferase inducibly in the entire mouse as shown by bioluminescence imaging (BLI) (ip injection with luciferin substrate and imaged 10 minutes later on a Xenogen Spectrum machine shows a colored region throughout). Dox – doxycycline was given to animals in the drinking water [2 mg/ml]. Animal 1 has a not been placed on Dox.
To: Dr. Phuoc Tran  
Department of Oncology  

From: Nancy A. Ator, Ph.D.  
Chair, Animal Care and Use Committee  

Date: 12/10/2010  

Subject: Amendment Approval Memo  

On 12/09/2010, the Johns Hopkins University Animal Care and Use Committee (ACUC) approved the following amendment to your research protocol. A copy of the approved amendment is attached.

Protocol Number: MO09M331

Title: Transgenic models of oncogene induced tumorigenesis and organ fibrosis

Expiration Date: 08/21/2011

Additional modifications to this protocol can be requested by submitting the appropriate amendment form (i.e., Change in Animal Number, Change in Personnel, or Change in Procedures) to the ACUC office for review and approval. Copies of all current forms can be found on our website: www.jhu.edu/animalcare.

For guidance on protocol modifications that require amendments, please refer to the reverse side of this letter. If the locations for outside housing or procedures change, please submit a Change in Location Form, also available on the website.
CHANGE IN PROCEDURE(S) OR ANIMAL NUMBERS
AMENDMENT REQUEST FORM

Release date: 12/08
Protocol Number: M00331

Protocol Title: Transgenic Models of Oncogene Induced Tumorigenesis and Organ Fibrosis
Principal Investigator: Phuoc T. Tran
Department: Radiation Oncology
Building: CRB2
Office Phone: x43880

School: SOM
Room: B406
Fax: x22821
Campus: East Baltimore
E-mail: tranp@jhmi.edu

If this request is being faxed or emailed (with an electronic signature) to the ACUC Office, an original is not needed.

Please indicate which changes you are requesting by an X next to each category below. Describe the change(s) and reasons on page 2 of this form. Please return a signed copy of this form to the ACUC Office, Reed Hall, room B122 or fax to 443-287-3747 (7-3747).

To add new personnel or change the PI, please complete the Change in Personnel Amendment Request Form or Change in PI Amendment Request Form.

To change a location for animal use complete the Change in Location Form.
All forms are available on the web at www.jhu.edu/animalcare/forms1.html.

Modify anesthetic or analgesic agents: State the name of the agent, dose or dose range, route of administration and frequency range for any drug to be added. Previously approved agents will remain on the protocol. If you need to withhold analgesia, indicate the reasons why and see “Modify Pain Category” below to see if it applies.

Modify Euthanasia: Describe any changes in the method of euthanasia (be sure proposed method is in compliance with the 2007 AVMA Guideline on Euthanasia, which can be viewed at www.avma.org/resources/euthanasia.pdf)

Modify Procedures: Provide a complete description and rationale for the proposed experimental changes. Indicate if they will change the degree of invasiveness of a procedure or discomfort to the animal. (i.e., the withholding of analgesia; change from non-survival to survival surgery; change in number, duration, or frequency of procedures performed on the animal, etc.). See “Modify Pain Category” below to determine if it applies.

Modify Surgical Procedures: Describe any changes to approved surgical procedures.

Modify Radiation; or Radioactive, Infectious or Biohazardous Agent: Provide rationale for adding this new agent, list all necessary safety precautions, and describe any modifications you plan to make to your currently-approved procedures. Attach pertinent approval letter or copy of application from Health, Safety & Environment as appropriate.

Modify Animal Numbers: Indicate the number of additional animals you are requesting that will fall under each pain category in the chart below. Provide a justification for the change in animal numbers. Each animal should be categorized only once. If adding animals or procedures to category D or E for the first time, please see “Modify Pain Category” below.

<table>
<thead>
<tr>
<th>Number Requested</th>
<th>Pain Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>B Breeder</td>
<td></td>
</tr>
<tr>
<td>C No pain or distress</td>
<td></td>
</tr>
<tr>
<td>D Alleviated Pain or distress</td>
<td></td>
</tr>
<tr>
<td>E Unalleviated Pain or distress</td>
<td></td>
</tr>
</tbody>
</table>

Revised 12/08
Procedure and Animal Numbers Amendment Form, Page 1
Modify Pain Category: Please describe the changes that will affect the pain category. If adding animals or procedures to category D or E for the first time, please include a description of what alternatives to procedures that may cause more than momentary or slight pain or distress have been considered and why no alternative was selected. See questions 17b-e on the full protocol form for the information that should be included with respect to category D or E procedures.

Add Satellite Housing: Include Satellite Housing amendment with this form

Other: describe on page 2.

CHANGE IN PROCEDURE(S)
AMENDMENT REQUEST FORM

Describe the requested change(s) following the guidelines for the specific modification as per page 1 of the form (attach additional pages as necessary).

To determine the role of oncogenes, such as ERG, for tumorigenesis and tumor maintenance using the Tet system.

Justification: Tumorigenesis is thought to involve multiple steps many of which are determined by changes in specific genes. Studies have demonstrated that oncogenes are causative in tumorigenesis. Oncogenes are also involved during normal developmental processes where cells acquire increased migratory abilities enabling cells to form the many and varied organ of the body. Dysfunctional oncogene expression has been implicated in both tumorigenesis and tissue fibrosis. The Tran laboratory is interested in understanding the role of various oncogenes, including but not limited to Twist1, hSNAI1 and ERG, in the processes of tumorigenesis, tumor maintenance and tissue fibrosis using mice that express oncogenes. In most cases, the expression of these oncogenes will be induced or turned “ON” and “OFF” using the tetracycline (or doxycycline) regulatory system (TET system).

Development of imaging surrogates for use in localization and monitoring treatment of tumors and organ fibrosis in living rodent subjects has been previously described in approved amendments. Many of the animal models we use are transgenic models (knock in, knock out) that recapitulate human disease. There are no computer simulations that serve this purpose.

We hypothesize that serial non-Invasive imaging followed by confirmation with histopathology will allow our group to monitor the development of tumors and track tumor regression in our cohort of transgenic mouse models using the Tet system.

1) To use non-invasive serial imaging studies and standard histo-pathological analysis to monitor tumorigenesis using the Tet system. We will determine if expression of oncogenes alone or in conjunction with previously approved agents and other oncogenes enhance tumorigenesis and/or lung fibrosis in the mice models as a part of our already approved protocol by providing the animals doxycycline in their water or chow as (MO09M331).

Cohorts of weaned, age-matched, control and experimental mice will be devoid of doxycycline or placed on doxycycline (depending on the transgenic model) in their drinking water to activate expression of an oncogene being studied. Mice will be monitored weekly for symptoms of morbidity as stated below. Prior experience with a separate luciferase tagged primary Twist1 tumor model indicates that bioluminescence imaging (BLI) signal correlates with tumor burden. Therefore, cohorts with the Luc reporter will also be followed for tumor development non-invasively by use of serial BLI (using our already approved imaging amendment) and correlated with disease pathology following necropsy at defined periods. Based on prior literature and our experience mice from each cohort will be sacrificed at time points of between 0-18 months of age depending on physical and imaging findings. These animals will be processed at necropsy for prostate lobes, other genitourinary (GU) organs, lungs, heart, liver and spleen and these specimens will be harvested for histology and flash frozen for molecular studies.

Revised 12/08
Procedure Amendment Form, Page 2
2) To use non-invasive serial imaging studies and standard histo-pathological analysis to monitor tumor maintenance using the Tet system.

Following development of autochthonous tumors in Tet regulated oncogene mice as determined by serial imaging and from my time course studies above, we will treat mice with doxycycline to simulate targeted treatment against the tetO-regulated oncogenes.

Tumor moribund mice that are known have tumors from imaging or suspected based on time course experiments above will be injected intraperitoneally with 100 micrograms of doxycycline in PBS and then restricted to water containing doxycycline changed weekly (or depending on the system normal water free of doxycycline). Cohorts of tumor morbid mice following oncogene inactivation will be followed by weekly inspection and imaging. At defined periods of between 0-12 month animals will serially imaged and sacrificed and necropsies and tumor analysis performed as above; or before if euthanasia is required for humane reasons.

All animals will be monitored and euthanized immediately if they exhibit the following symptoms:
- Ulceration and bleeding of the tumor
- Anorexia indicated by the absence of feces in cage
- Does not drink water leading to dehydration evidenced by tenting of the skin
- Hunched up, unwilling to move, favoring a limb or guarding the incision site
- Failure to groom reflected in a ruffled or dirty coat
- Excessive licking/scratching, redness and swelling at incision site, and self-mutilation
- Aggressive behavior especially when attempting to pick up the animal
- Squealing, struggling, twitching, tremors, convulsions, weakness
- Panting, labored breathing, reddish-brown nasal/ocular discharge
- Cold or blue extremities (hypothermia) or hot or red extremities (hyperthermia)

I understand that these changes must not be implemented until I receive approval for the changes from the Animal Care and Use Committee.

PI Signature: ______________________________ Date: 11/18/2010

IACUC Chair's Signature: ______________________________ Date: 12/9/10

Revised 12/08
Procedure Amendment Form, Page 2