TITLE: Evaluating the efficacy of ERG targeted therapy in vivo for prostate tumors

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# Evaluating the efficacy of ERG targeted therapy in vivo for prostate tumors

## Abstract
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 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 fifth 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. To remedy this issue, we re-started Task #1 two years ago with the new prostate specific TET driver mouse, Hoxb13-rtTA. We have spent this last year examining whether ERG can collaborate with AKT1 with these new mice, Hoxb13-rtTA/tetO-ERG. We had to reinitiate breeding of more mice using a different tetO-ERG founder line and are in the midst of processing samples for analysis. Despite these setbacks, 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 and international recognition.

## Subject Terms
ERG, prostate cancer, inducible transgenic mouse model
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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 1-4 and BOLD font for the past year (Year 5).

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 1-4 Progress Reports, 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).

We had to get re-approval of our IACUC protocol this past year (see Appendix)

1a. Mating mice to characterize (months 4-10).
As reported in our Year 1-4 Progress Reports, the appropriate single transgene ARR2PB-rTA (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 stated in the last progress report (Year 4) that we were generating more 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 characterized these HE mice using as a reporter, functional expression of luc detected with bioluminescence imaging (BLI) and molecular characterization with qPCR and Western for ERG (data not shown; see Table 1B and Table 2B). We did 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 was 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 another founder line of 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 no shown). The possible explanations are that the HE transgenic combination we selected was not compatible or the particular tetO-ERG founder line we used may have undergone silencing which is a well characterized phenomenon in transgenic mice. We switched to a different tetO-ERG founder line and began to generate more bitransgenic, Hoxb13-rtTA/ERG-tetO-luc (HE) and tritransgenic Hoxb13-rtTA/MPAKT1/ERG-tetO-luc (HA1E) mice to perform Task #1 and Task #2, respectively.

1b. Collecting tissues from AE mice to characterize 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 1-4 Progress Reports, the appropriate numbers of AE bitransgenic mice (n=25) had been placed on drinking water without doxycycline to activate the ERG transgene.

We collected tissues from HE mice with and without doxycycline (Table 1B). We will proceed with this sub-Task #1b again when we get more animals with a new founder tetO-ERG line (see Task #1a above).

1c. Collecting tissues from AE mice turned OFF to characterize inducible ERG-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 1-4 Progress Reports, 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.

We collected tissues from HE mice turned OFF (Table 2B). We will proceed with this sub-Task #1c again when we get more animals with a new founder tetO-ERG line (see Task #1a above).

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 AE mice 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.

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 & Tables 1B and 2B below).

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.

We performed some analysis on the HE mice as summarized below in Table 1B & 2B and we do not observe any gross abnormalities in any of the samples. Nor did we observe any expression of ERG by BLI or other molecular techniques.

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>
<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>
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<tbody>
<tr>
<td>AE</td>
<td>6 mice</td>
<td>7 mice</td>
<td>5 mice</td>
<td>5 mice</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>ND</td>
<td>ND</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>

A – ARR2PB; DOX – doxycycline; E – luc-tetO-ERG; IHC – immunohistochemistry; qPCR – quantitative polymerase chain reaction; WNL – within normal limits.

Table 1B – Summary of Task #1b & d 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>6 mice</td>
<td>6 mice</td>
<td>5 mice</td>
</tr>
<tr>
<td>Gross</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
<td>ND</td>
</tr>
<tr>
<td>Histologic</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
<td>ND</td>
</tr>
<tr>
<td>Myc IHC</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FLAG IHC</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FLAG Western</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ERG qPCR</td>
<td>Negative expression</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

A – ARR2PB-tTA; DOX – doxycycline; E – luc-tetO-ERG; IHC – immunohistochemistry; qPCR – quantitative polymerase chain reaction; WNL – within normal limits.

Table 2A – Summary of Task #1c & d to date.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>1 wks Off DOX</th>
<th>2 wks Off DOX</th>
<th>4 wks Off DOX</th>
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<tbody>
<tr>
<td>AE</td>
<td>6 mice</td>
<td>6 mice</td>
<td>6 mice</td>
</tr>
<tr>
<td>Gross</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
</tr>
<tr>
<td>Histologic</td>
<td>WNL</td>
<td>WNL</td>
<td>WNL</td>
</tr>
<tr>
<td>Myc IHC</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FLAG IHC</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FLAG Western</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ERG qPCR</td>
<td>Negative expression</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

A – ARR2PB-tTA; DOX – doxycycline; E – luc-tetO-ERG; IHC – immunohistochemistry; qPCR – quantitative polymerase chain reaction; WNL – within normal limits; ND - not done.

Table 2B – Summary of Task #1c & d to date.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>1 wks Off DOX</th>
<th>2 wks Off DOX</th>
<th>4 wks Off DOX</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>3 mice</td>
<td>3 mice</td>
<td>ND</td>
</tr>
<tr>
<td>Gross</td>
<td>WNL</td>
<td>WNL</td>
<td>ND</td>
</tr>
<tr>
<td>Histologic</td>
<td>WNL</td>
<td>WNL</td>
<td>ND</td>
</tr>
<tr>
<td>Myc IHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FLAG IHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IHC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

A – ARR2PB-tTA; DOX – doxycycline; E – luc-tetO-ERG; IHC – immunohistochemistry; qPCR – quantitative polymerase chain reaction; WNL – within normal limits; ND - not done.
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).
We bred Hoxb13-rtTA/MPAKT1/ERG-tetO-Luc (HA1E) mice for Task #2a, but aborted and sacrificed these specific HA1E mice for the reasons as explained above in Task #1d. We have re-initiated the mating required to produce Hoxb13-rtTA/MPAKT1/ERG-tetO-Luc (HA1E) mice using a new founder tetO-ERG line.

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 is dependent on the steps above and has 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.
• Characterization of the HE transgenic mouse showed that either the transgene combination used was not compatible or this particular tetO-ERG founder line we used may have undergone silencing, which is a well characterized phenomenon in transgenic mice.

• Re-initiated breeding for 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 and a new founder tetO-ERG line.

5. CONCLUSION:
During this fifth and last year of support we have not been able to 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 have been 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).

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 low level of expression from the ARR2PB-tTA line 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 re-start 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 as compared to our original ARR2PB-tTA line (see Figure 1 Appendix).

In Years 3-4, the breeding between our tetO-ERG mice and Dr. Bieberich’s Hoxb13-rtTA mice had been problematic, but we overcame these issues and were able to proceed with Tasks #1a-c with the Hoxb13-rtTA/tetO-ERG (HE) mice (see Table 1B & 2B). We also started breeding mice for Task #2a, but aborted and sacrificed these specific HA1E mice for the reasons below.

In this final Year 5, we completed Task #1d with the HE mice and continued with breeding HA1E mice for Task #2 studies. Unfortunately, we did not observe expression with BLI, via qPCR for ERG or Western for ERG in HE mice (see Table 1B & 2B). We have troubleshooting this situation and believe the specific HE transgenic mouse combination we used was not compatible or this particular tetO-ERG line we used may have undergone silencing which is a well characterized phenomenon in transgenic mice. We are in the process of breeding with a new founder tetO-ERG line more HE mice (Task #1a) to proceed again with Tasks #1b-d.

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 and international 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 AKT1 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 and international recognition.
6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS:

- During this fifth and last 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:
   We were awarded a Movember-PCF Challenge grant to test a novel radiation and immunotherapy combination in oligometastatic prostate cancer patients.

   We were noticed for a donation to our prostate cancer research efforts by a local philanthropic group.

Coverage of a collaborative study where we developed a novel small animal optical imaging platform for preclinical research.

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.


11. Emelyn H. Shroff, Livia S. Eberlin, Vanessa M. Dang, Arvin M. Gouw, Meital Gabay, Stacey J. Adam, David I. Bellovin, **Phuoc T Tran**, William M. Philbricke, Adolfo Garcia-Ocanaf, Stephanie C. Casey, Yulin Li, Chi V. Dang, Richard Zare, Dean W. Felscher. MYC Oncogene Overexpression Drives Renal Cell Carcinoma in a Mouse Model through Glutamine


* - these authors contributed equally.
3. Invited Articles (Since the beginning of the DoD PRTA):


4. Abstracts (Year 5 only from a total of 47 since the beginning of the DoD PRTA):


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”.
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”.

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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”.
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”.
22. JHU 38th Biennial Meeting & Reunion – SKCCC Seminar (June 5, 2015 – Baltimore, MD). “Altering the Natural History of Oligometastatic Prostate Cancer with Local Ablative Therapies”.
24. PCF Norway Prostate Cancer Symposium 2015 – Accelerating Innovations and Advancing Discovery (June 12, 2015 – Oslo, Norway). “Advanced Radio-therapeutic approaches to manage Prostate Cancer - Proton Beam - Does this costly technology improve outcomes?”.
25. PCF Coffey-Holden Prostate Cancer Academy Meeting (June 28, 2015 – La Jolla, CA). “Metastasis Directed Therapy – The Radiation Oncologist’s Perspective”.
27. Movember Foundation Visiting Professor (October 13, 2015 – Melbourne, Australia). “Stereotactic Ablative Techniques for Oligometastatic Prostate Cancer”.
28. Clemenceau Medical Center - Cancer Forum (October 30, 2015 – Beirut, Lebanon). “Oligometastatic disease: paradigm shift from palliative approach to curative approach”.
29. University of Chicago, Radiation & Cellular Oncology (February 12, 2016 – Chicago, IL). “Metastatic Prostate Cancer – Basic to Clinical Interrogation”.

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

8. REPORTABLE OUTCOMES:
Nothing to report.

9. OTHER ACHIEVEMENTS:

CLINICAL TRIALS:
2. J1153 - Pharmacodynamic Trial of Pre-Prostatectomy Lovastatin on MYC Down-Regulation in Localized Prostate Cancer. Role: PI. Closed early.


6. J15180 - Phase II Randomized Observation versus Stereotactic Ablative Radiation for Oligometastatic Prostate Cancer (ORIOLE) trial. Role: PI. Open to accrual.


8. URO-004 - A Retrospective Study of Prolaris® for the Prediction of Progression in Men Treated with Modern External Beam Radiation Therapy for Prostate Cancer. Role: PI. Under IRB review.

GRANTS:
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

3. Movember-PCF Challenge Tran (PI) 8/1/15-7/30/2017
   Movember-Prostate Cancer Foundation (PCF)
   “Altering the Natural History of Metastatic Prostate Cancer using Stereotactic Ablative Radiotherapy (SABR) and Immune Stimulation”
   The overall goal is to test consolidation of all sites of macroscopic disease with SABR in combination with the immune stimulatory agent ADXS-PSA in men with oligometastatic prostate cancer using a first-in-man clinical trial and complimentary correlative approaches.
   Role: PI

4. Patient-Centered Outcomes Res Smith (PI) 11/1/15-10/31/20
   Patient-Centered Outcomes Research Institute (PCORI)
   Simplifying Survivorship Care Planning: Comparing the Efficacy and Patient-Centeredness of Three Care Delivery Models in Prostate, Breast and Colorectal Cancer
   Role: Co-I
COMPLETED:

1. 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

2. 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

3. 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

4. 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:
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.
2015-2016  Johns Hopkins Catalyst Award.
2015-2017  Movember Foundation-Prostate Cancer Foundation (PCF) Challenge Award.

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

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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  Swiss and French Prostate Cancer Foundation-Movember Foundation, Ad Hoc Scientist Reviewer
2016  Flemish Cancer Society Kom op tegen Kanker (SU2C), Ad Hoc Scientist Reviewer
2016-2018  RSNA R&E Foundation – Radiation Oncology Research Study Section, Chair
2016-2021  NIH NCI RTB R01, Permanent Reviewer

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 V - Translational Radiobiology, 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
2015  ASTRO National Meeting, Session EE - Biology V - Imaging and Circulating Biomarkers, Co-Chair
2016  3rd Amtrak Alliance - Baltimore-Philadelphia Prostate Cancer Summit – Localized Prostate Cancer, Chair
2016  RRS National Meeting, Symposium – Lung Cancer, Chair
2017  ASTRO-NCI Immunotherapy and Radiation Oncology Workshop, Co-Chair

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.
Dr. Phuoc Tran  
Department of Oncology

Dear Dr. Tran:

On 07/17/2015, the Johns Hopkins University Animal Care and Use Committee (ACUC) approved the following research protocol for which you are the Principal Investigator. A copy of the approved protocol is attached.

Protocol Number: MO15M273

TITLE: Transgenic models of oncogene induced tumorigenesis and organ fibrosis (replaces MO12M261)

The approval period is for three (3) years. The ACUC office will send a notice reminding you to submit the 3rd year replacement protocol. This notice will be sent out 90 days prior to the expiration date. Please use this protocol number when placing an order with Research Animal Resources (RAR) (formerly known as Animal Services). They can be contacted by calling 5-3713. Note: Approval of this protocol does not guarantee University space for housing animals.

You may request modifications to this protocol 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 our 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.

Sincerely,

[Signature]

Nancy A. Ator, PhD  
Chair, Animal Care and Use Committee