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14. 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 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 *ARR2PB-tTA* line lead us to conclude that the level of expression was insufficient for *in vivo* experimentation. To remedy this issue, we re-started Task #1 last year with the new prostate specific TET driver mouse, *Hoxb13-rtTA*. We have spent this last year reinitiating our studies on the ability of ERG to collaborate with *AKT1* with these new mice, *Hoxb13-rtTA*/*tetO-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.

15. 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 1-3 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 no 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 $\geq$ 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 Tabe 2 for summary of results and "Conclusions" below for explanation of results.

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

Genotype	4 wks On DOX	8 wks On DOX	12 wks On DOX	24 wks On DOX
AE	6 mice	7 mice	5 mice	Pending
Gross	WNL	WNL	WNL	WNL
Histologic	WNL	WNL	WNL	WNL
Myc IHC	Negative expression	Negative expression	Negative expression	Negative expression
FLAG IHC	Negative expression	Negative expression	Negative expression	Negative expression
luc IHC	ND	ND	ND	Negative expression
FLAG Western	Negative expression	Negative expression	ND	Negative expression
***ERG* qPCR**	ND	ND	ND	Negative expression

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

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

Genotype	4 wks On DOX	8 wks On DOX	12 wks On DOX	24 wks On DOX
HE	4 mice	Pending	Pending	Pending
Gross	WNL	Pending	Pending	Pending
Histologic	WNL	Pending	Pending	Pending
Myc IHC	Pending	Pending	Pending	Pending
FLAG IHC	Pending	Pending	Pending	Pending

luc IHC	Pending	Pending	Pending	Pending
FLAG Western	Negative expression	Pending	Pending	Pending
***ERG* qPCR**	Negative expression	Pending	Pending	Pending

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 to date.

Genotype	1 wks Off DOX	2 wks Off DOX	4 wks Off DOX
AE	6 mice	6 mice	Pending
Gross	WNL	WNL	WNL
Histologic	WNL	WNL	WNL
Myc IHC	Negative expression	ND	ND
FLAG IHC	Negative expression	ND	ND
luc IHC	Negative expression	ND	ND
FLAG Western	Negative expression	ND	ND
***ERG* qPCR**	Negative expression	ND	ND
IHC	Negative expression	ND	ND
Western	Negative expression	ND	ND

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 to date.

Genotype	1 wks Off DOX	2 wks Off DOX	4 wks Off DOX
HE	3 mice	Pending	Pending
Gross	WNL	Pending	Pending
Histologic	WNL	Pending	Pending
Myc IHC	Pending	Pending	Pending
FLAG IHC	Pending	Pending	Pending
luc IHC	Pending	Pending	Pending
FLAG Western	Pending	Pending	Pending
***ERG* qPCR**	Pending	Pending	Pending
IHC	Pending	Pending	Pending
Western	Pending	Pending	Pending

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).
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 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 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 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 *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 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):

     1. **Phuoc T. Tran**+, Russell K. Hales, Jing Zeng, Khaled Aziz , Tarek Salih, Rajendra P. Gajula, Sivarajan Chettiar, Nishant Gandhi, Aaron T. Wild, Rachit Kumar, Joseph M. Herman, Danny Song and Theodore L. DeWeese. Tissue Biomarkers for Prostate Cancer Radiation Therapy. ***Curr Mol Med*** 12 (2012) 772-787. PMID: 22292443; PMCID: PMC3412203.
        + - corresponding author.

2. Nishant Gandhi*, Aaron T. Wild*, Sivarajan T. Chettiar, Khaled Aziz, Yoshinori Kato, Rajendra P. Gajula, Russell D. Williams, Jessica Cades, Anvesh Annadanam, Danny Song, Yonggang Zhang, Russell K. Hales, Joseph M. Herman, Theodore L. DeWeese, Edward M. Schaeffer, **Phuoc T. Tran**. Novel Hsp90 inhibitor NVP-AUY922 radiosensitizes prostate cancer cells. ***Cancer Biol Ther*** 14 (2013) 347-356. PMID: 23358469. PMCID: PMC3667875.
   * - these authors contributed equally.
3. Jason A. Efstathiou, Deborah S. Nassif, Todd R. McNutt, C. Bob Bogardus, Walter Bosch, Jeff Carlin, Ron C. Chen, Henry Chou, Dave Eggert, Benedick Fraass, Joel Goldwein, Karen E. Hoffman, Ken Hotz, Margie Hunt, Marc Kessler, Colleen A. F. Lawton, Chuck Mayo, Jeff M. Michalski, Sasa Mutic, Louis Potters, Chris M. Rose, Howard M. Sandler, Greg Sharp, Wolfgang Tomé, **Phuoc T. Tran**, Terry Wall, Anthony L. Zietman, Peter E. Gabriel, Justin E. Bekelman. Practice-Based Evidence to Evidence-Based Practice: Building the National Radiation Oncology Registry. ***J Oncol Pract*** 9 (2013) e90-95. PMID: 23942508. PMCID: PMC3651578.
4. Sara Alcorn*, Amanda J. Walker*, Nishant Gandhi, Amol Narang, Aaron T. Wild, Russell K. Hales, Joseph M. Herman, Danny Y. Song, Theodore L. DeWeese, Emmanuel Antonarakis, **Phuoc T. Tran**. Molecularly Targeted Agents as Radiosensitizers in Cancer Therapy – Focus on Prostate Cancer. ***Int J Mol Sci*** 14 (2013) 14800-14832. PMID: 23863691. PMCID: PMC3742274.
   * - these authors contributed equally.
5. Rajendra P. Gajula*, Sivarajan T. Chettiar*, Russell D. Williams, Saravanan Thiyagarajan, Yoshinori Kato, Khaled Aziz, Ruoqi Wang, Nishant Gandhi, Aaron T. Wild, Farhad Vesuna, Jinfang Ma, Tarek Salih, Jessica Cades, Elana Fertig, Shyam Biswal, Timothy F. Burns, Christine Chung, Charles M. Rudin, Joseph M. Herman, Russell K. Hales, Venu Raman, Steven An, **Phuoc T. Tran**. The twist box domain is required for Twist1-induced prostate cancer metastasis. ***Mol Cancer Res*** 11 (2013) 1387-1400**. PMID: 23982216. PMCID: PMC3833995.
   * - these authors contributed equally.
   ** - Cover illustration and Highlighted in *Mol Cancer Res.*
6. Debasish Sundi, Vinson Wang, Phillip M. Pierorazio, Misop Han, Alan W. Partin, **Phuoc T. Tran**, Ashley E. Ross, Trinity J. Bivalacqua. Identification of men with the highest risk of early disease recurrence after radical prostatectomy. ***Prostate*** 74 (2014) 628-36. PMID: 24453066; PMCID: PMC4076164.
7. Minh-Phuong Huynh-Le, Zhe Zhang, **Phuoc T. Tran**, Theodore L. DeWeese, Daniel Y. Song. Low inter-rater reliability in grading of rectal bleeding using NCI-CTC and RTOG toxicity scales: a survey of radiation oncologists. ***Int J Radiat Oncol Biol Phys*** 90 (2014) 1076-1082. PMID: 25442040. PMCID: PMC4276525.
8. Rajendra P. Gajula*, Sivarajan T. Chettiar*, Russell D. Williams, Katriana Nugent, Yoshinori Kato, Hailun Wang, Reem Malek, Kekoa Taparra, Jessica Cades, Anvesh Annadanam, A-Rum Yoon, Elana Fertig, Beth A. Firulli, Lucia Mazzacurati, Timothy F. Burns, Anthony B. Firulli, Steven An, **Phuoc T. Tran**. Structure-function studies of the bHLH phosphorylation domain of Twist1 in prostate cancer cells. ***Neoplasia*** 17 (2015) 16-31. PMID: 25622896. PMCID: PMC4309734.
   * - these authors contributed equally.
9. Lynnette R Ferguson, Helen Chen, Andrew R. Collins, Marisa Connell, Giovanna Damia, Santanu Dasgupta, Meenakshi Malholtra, Alan K Meeker, Amedeo Amedei, Amr Amin, S. Salman Ashraf, Katia Aquilano, Asfar S. Azmi, Dipita Bhakta, Alan Bilsland, Chandra S. Boosani, Sophie Chen, Maria Rosa Ciriolo, Hiromasa Fujii, Gunjan Guha, Dorota Halicka, William G. Helferich, W. Nicol Keith, Sulma I. Mohammed, Elena Niccolai, Xujuan Yang, Kanya Honoki, VirginiaR. Parslow, Satya Prakash, Sarallah Rezazadeh, Rodney E Shackelford, David Sidransky, **Phuoc T Tran**, Eddy S. Yang, and Christopher A Maxwell. Genomic

instability in human cancer: molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. ***Semin Cancer Biol*** (2015). *In press.* PMID: 25869442.

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

1. **Phuoc T. Tran**, Trinity J. Bivalacqua, Adam P. Dicker. Adjuvant Radiation for Node Positive Disease Post-Prostatectomy - More Good News But Who Will Listen? ***J Clin Oncol*** 32 (2014) 3917-3919. PMID: 25311219.
2. **Phuoc T. Tran**, Trinity J. Bivalacqua, Adam P. Dicker. Reply to CG Rusthoven et. al. ***J Clin Oncol*** (2015) *In press.* PMID: 25847932.

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.
2. Narang AK, Ram AN, Robertson S, Hei P, Griffith E, Singh H, DeWeese, TA, Honig, S, McNutt T, Song DY, **Tran PT**, DeWeese TL. End of treatment PSA as a novel prognostic factor in patients undergoing definitive radiation for prostate cancer. ASTRO 2014 National Meeting.
3. Ram AN, Robertson S, Narang AK, Hei P, Griffith E, Singh H, DeWeese TA, Honig S, McNutt T, DeWeese TL, Song DY, **Tran PT**. Prognostic value of PSA nadir in patients undergoing definitive radiation for prostate cancer. ASTRO 2014 National Meeting.
4. Robertson S, Narang AK, Ram AN, He P, Griffith E, Singh H, DeWeese TA, Honig S, McNutt T, **Tran PT**, DeWeese TL, Song DY. Prognostic and therapeutic implications of PNI in prostate cancer patients undergoing definitive radiation. ASTRO 2014 National Meeting.
5. Narang AK, Robertson S, Ram AN, He P, Sundi D, Griffith E, Singh H, DeWeese TA, Honig S, McNutt T, Ross AE, Bivalacqua TJ, Schaeffer EM, Partin AW, DeWeese TL, **Tran PT**, Song DY. Very-high-risk localized prostate cancer – outcomes following definitive radiation. **Oral presentation** for the ASTRO 2014 National Meeting.
6. Ram AN, Narang AK, Robertson S, He P, Sundi D, Griffith E, Singh H, DeWeese TA, Honig S, McNutt T, Schaeffer EM, Ross AE, Bivalacqua TJ, Partin AW, Song DY, **Tran PT**, DeWeese TL. Multiple intermediate-risk factors as a prognostic tool for men with localized prostate cancer. ASTRO 2014 National Meeting.
7. Minh-Phuong Huynh-Le, Zhe Zhang, **Phuoc Tran,** Theodore DeWeese, Danny Y Song. Discrepancies in toxicity grading of rectal bleeding: a survey of radiation oncologists who treat prostate cancer. 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.
9. Andrew J. Armstrong, Susan Halabi, Patrick Healy, Robert Lee, Bridget F. Koontz, Judd W. Moul, Kelly Mundy, Patricia Creel, Sarah Yenser Wood, Kristen Davis, Brooke Reimer, Minh Nguyen, Avery N. Spitz, Ellen Bratt, Sung Kim, **Phuoc T. Tran**, Mark N. Stein, Michael Anthony Carducci, Daniel J. George. A phase 2 multimodality trial of docetaxel/prednisone with sunitinib followed by salvage radiation therapy (RT) in men with PSA recurrent prostate cancer (PC) after radical prostatectomy (RP). Genitourinary Cancers Symposium 2015.
10. **Tran PT**, Narang AK, Ram AN, Robertson S, Hei P, Griffith E, Singh H, DeWeese, TA, Honig, S, McNutt T, Song DY, DeWeese TL. End of radiation PSA as a novel prognostic

factor in patients undergoing definitive radiation for prostate cancer. Genitourinary Cancers Symposium 2015.

11. Daniel Y. Song, Amol Narang, Scott P. Robertson, Ashwin Ram, Pei He, Debasish Sundi, Emily Griffith, Harleen Singh, Alex DeWeese, Stephanie Honig, Todd R. McNutt, Ashley Ross, Trinity Bivalacqua, Edward M. Schaeffer, Alan W. Partin, Theodore L. DeWeese, **Phuoc T. Tran**. Very-high-risk localized prostate cancer – outcomes following definitive radiation. Genitourinary Cancers Symposium 2015**.**
12. Debasish Sundi, Jason Cohen, Vinson Wang, Mark W Ball, Farzana A Faisal, Amol Narang, **Phuoc Tran**, Danny Song, Theodore DeWeese, Ashley E Ross, Trinity J Bivalacqua, Edward M Schaeffer. Pre-treatment distinction of low-intermediate- from high-intermediate-risk among men with localized prostate cancer. American Urological Association Annual Meeting 2015.

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".
4. JHU, Brady Urology Prostate Cancer Research Day (February 25, 2012). "Using High-Dose Statins to Target MYC-overexpressing Prostate Cancers".
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".
8. Baltimore-Philadelphia Prostate Cancer Summit (November 1, 2013). "Phase I Trial of HSP90 inhibition and radiation-androgen deprivation therapy for high-risk, localized and locally advanced prostate cancer".
9. 6th Biennial TEMTIA Meeting: Symposium I – Cell/Molecular Biology of EMT (November 13, 2013). "The Twist box domain is required for TWIST1-induced metastasis of prostate cancer cells".
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".
12. Delaware Society for Clinical Oncology (May 22, 2014). "Emerging genetic tests for localized prostate cancer: ready for prime time?".
13. Prostate Cancer UK 11th Biennial Prostate Cancer Forum (June 13, 2014). "SBRT/SBAR for Oligomets".
14. Amtrak Alliance - Baltimore-Philadelphia Prostate Cancer Summit (November 7, 2014). "SBRT/SBAR for Oligomets".
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".

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):

1. J0910 - Multimodality Therapy for Recurrent High Risk Prostate Cancer: A Phase II Study. Role: PI. *Closed early.*
2. J1153 - Pharmacodynamic Trial of Pre-Prostatectomy Lovastatin on MYC Down-Regulation in Localized Prostate Cancer. Role: PI. *Closed early*.
3. J11157 - Stereotactic Body Radiation Therapy and Short-Term Androgen Ablation for Intermediate-Risk, Localized, Adenocarcinoma of the Prostate. Role: PI. *Open to accrual.*
4. J1454 – SALVENZA Trial: Phase II Randomized Placebo-Controlled Double-Blind Study of Salvage Radiation Therapy (SRT) Plus Placebo *Versus* SRT Plus Enzalutamide in Men with High-Risk PSA-Recurrent Prostate Cancer after Radical Prostatectomy. Role: PI. *Open to accrual.*
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
2011-2013 NASA Space Radiation Cancer Risks - Ground-Based Studies in Space Radiobiology, Panel Reviewer
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 The Halifax Project Task Force: A Broad-Spectrum Integrative Design for Cancer Prevention and Therapy – Genetic Instability Group
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 NIH NCI Loan Repayment Program Special Emphasis Panel - ZCA1 PCRB-A (A2) S, 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 ORAGANIZER, 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

EDITORIAL ACTIVITIES (Since the beginning of the DoD PRTA):

2014- Cancer Research, Associate Editor – Breaking Advances

10. REFERENCES:

1. A. Jemal *et al.*, *CA Cancer J Clin* **59**, 225 (Jul-Aug, 2009).
2. C. Kumar-Sinha, S. A. Tomlins, A. M. Chinnaiyan, *Nature reviews* **8**, 497 (Jul, 2008).
3. S. A. Tomlins *et al.*, *Science* **310**, 644 (Oct 28, 2005).
4. V. Rao *et al.*, *Prostate*, (Feb 1, 2012).

11. APPENDIX:

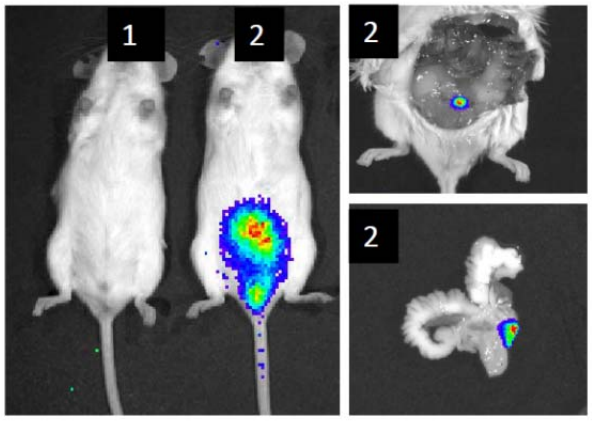

Fig 1. ***Generation of an inducible luc prostate epithelial specific mouse model.*** *Mice containing a prostate specific TET driver transgene, Hoxb13-rtTA was crossed with a reporter mouse luc-tetO-Twist1 line to produce bi-transgenic animals (HT). The presence of doxycycline allows the rtTA protein 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-rtTA 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.*

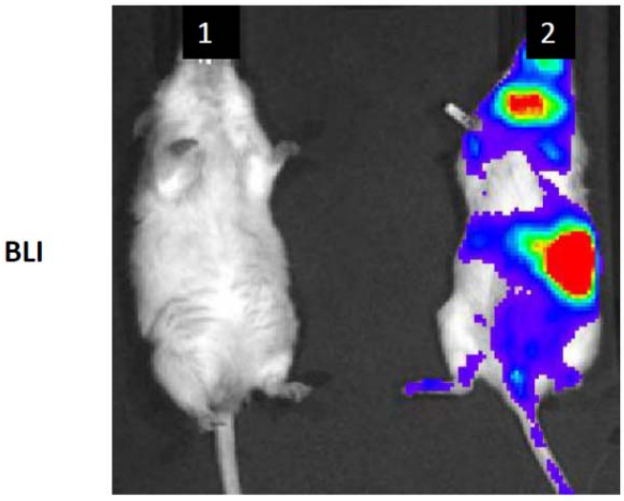

Fig 2. ***Generation of an inducible luc-tetO-ERG mouse model.*** *Mice containing a ubiquitous TET driver transgene, CMV-rtTA were crossed with our ERG line of interest, luc-tetO-ERG, that has the luc reporter to generate bi-transgenic animals (CMV-E). The presence of doxycycline allows the rtTA protein 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.*

JOHNS HOPKINS
UNIVERSITY

Animal Care and Use Committee

1620 McFiderry Street
Reed Hall, Room B122
Baltimore, Maryland 21205-1911
(443) 287-3738 / FAX (443) 287-3747
www.jhu.edu/animalcare

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 [Procedures amendment to your research protocol. A copy of the approved amendment is attached.

Protocol Number: MO09M331

Title: Transgenic models of oncogene induced tumorgenesis 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.

Johns Hopkins University Animal Care and Use Committee

CHANGE IN PROCEDURE(S) OR ANIMAL NUMBERS AMENDMENT REQUEST FORM

Release date: 12/08

Below for ACUC Use	
Date Received:	11/18/10
Expiration Date:	8/21/11
☑ Logged	☑ Database

Protocol Number: MO09331

Protocol Title: Transgenic Models of Oncogene Induced Tumorigenesis and Organ Fibrosis

Principal Investigator: Phuoc T. Tran

Department: Radiation Oncology School: SOM

Building: CRB2 Room: B406 Campus: East Baltimore

Office Phone: x43880 Fax: x22821 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)*

__X__ **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 analgesics; 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.*

Number Requested	*Pain Category*
	B Breeders
	C No pain or distress
	D Alleviated Pain or distress
	E Unalleviated Pain or distress

_____ **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 organs 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 doxcycline 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 biolumeniscence 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.

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: Nancy A. Aton Date: 12/9/10