

AD_____

Award Number:

W81XWH-10-1-0582

TITLE:

ETS Gene Fusions as Predictive Biomarkers of Resistance to
Radiation Therapy for Prostate Cancer

PRINCIPAL INVESTIGATOR:

Felix Feng, M.D.

CONTRACTING ORGANIZATION:

University of Michigan

Ann Arbor, MI 48109

REPORT DATE:

August 2013

TYPE OF REPORT:

Annual Summary

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE August 2013		2. REPORT TYPE Annual		3. DATES COVERED 15 July 2012 to 14 July 2013	
4. TITLE AND SUBTITLE ETS Gene Fusions as Predictive Biomarkers of Resistance to Radiation Therapy for Prostate Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-1-0582	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Felix Feng, M.D. E-Mail: ffeng@med.umich.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Michigan Ann Arbor, MI 48109-1340				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT- The research goals of this grant proposal are to: 1) investigate the effect of ETS gene fusions on radiation phenotype in preclinical models of prostate cancer, 2) to explore the mechanism of interaction between ERG (the predominant ETS gene fusion product) and the DNA repair protein DNA-PK, and 3) to determine if ETS gene fusion status is a clinical biomarker of radioresistance for prostate cancer. The training goals of this grant proposal included a series of regular meetings with mentors, research seminars, journal clubs, and workshops, all of which are intended to help Dr. Feng develop as a translational scientist. This grant proposal was approved as a five-year award; the current annual report summarizes accomplishments over the third year of the grant, from July 15, 2012 to July 15, 2013. Overall, the first three years of this grant have been very successful. The work accomplished as a result of this grant resulted in three publications in very high-impact journals (including a fourth accepted pending minor revisions), four presentations, and four grants (two from the Prostate Cancer Foundation, one from Celgene, and one from the Fund for Cancer Research). Additionally, Dr. Feng has met the training achievements specified in his original grant. The research proposed in this training grant represents an important area within the field of prostate cancer research. Because ETS gene fusions are thought to be driver alterations in over half of all prostate cancers, understanding the mechanistic and potential clinical implications of these gene fusions has significant ramifications, particularly in the context of radiation therapy, which represents a primary treatment modality for localized prostate cancer. In the third year of this grant period, we have accomplished another three subaims, for a total of 15 out of 20 originally proposed subaims accomplished over the first three years of this grant. These three subaims included two aimed at studying the interaction between ETS fusions and the DNAPK protein, as well as a third towards our biomarker goals. This work builds on our findings from our first two years that ERG (the predominant ETS gene fusion product) confers radioresistance in preclinical models of prostate cancer and that this radioresistance can be reversed with DNA-PK inhibition. In total, our findings suggest that DNA-PK inhibition should be explored as a clinical strategy for radiosensitizing prostate cancers.					
15. SUBJECT TERMS- Prostate cancer, ETS gene fusions, ERG, radiation resistance, DNAPK					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	5
Body.....	5
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusion.....	9
References.....	11
Appendices.....	11

Introduction

This annual report will summarize the accomplishments associated with the Department of Defense Physician Research Training Award (W81XWH-10-1-0582), awarded to Felix Feng, M.D. This award included both research goals and training goals. The research goals of this grant proposal are to: 1) investigate the effect of ETS gene fusions on radiation phenotype in preclinical models of prostate cancer, 2) to explore the mechanism of interaction between ERG (the predominant ETS gene fusion product) and the DNA repair protein DNA-PK, and 3) to determine if ETS gene fusion status is a clinical biomarker of radioresistance for prostate cancer. The training goals of this grant proposal included a series of regular meetings with mentors, research seminars, journal clubs, and workshops, all of which are intended to help Dr. Feng develop as a translational scientist, with the ultimate goals of submitting a NIH-level grant as an independent investigator and developing a translational clinical trial. This grant proposal was approved as a five-year award; the current annual report summarizes accomplishments over the third year of the grant, from July 15, 2012 to July 15, 2013.

Body

Research achievements: Tasks and Subtasks

As outlined in the original Statement of Work, this grant proposal was comprised of three specific aims, subdivided into 7 tasks, which were further divided into 20 subtasks. In year 1, I accomplished seven subtasks (1A, 1B, 3A, 3B, 4A, 4B, and 4C), resulting in completion of Tasks #1 and #3. In year 2, I performed subtasks 2A, 2B, 6A, 7A, and 7B, resulting in progress in Tasks #2, #6, and #7. In year 3, I was able to complete an additional three subtasks (5A, 5B, and 6B), resulting in progress in Tasks #5 and #6. In total, I have now completed 15 out of 20 proposed subtasks over the first three years of my grant, which puts me ahead of the schedule outlined in my initial statement of work (12 subtasks to be completed over the first 3 years). The findings associated with these subtasks and tasks from year 3 are detailed below.

The goal of Task #5 was to characterize the timing, location, and order of recruitment of the ERG:DNAPK interaction in relation to radiation delivery. Specifically, Task #5 included two subtasks. Subtask #5A was to make fusion constructs of ERG and DNAPK linked to different fluorescent proteins, and Subtask #5B was to overexpress these constructs in the VCaP cell line and perform real-time confocal microscopy to characterize the timing, location, and order of recruitment of the ERG-DNAPK interaction in relation to radiation delivery. In the first half of year 3, we generated fusion constructs of ERG to various fluorescent proteins [green fluorescent protein (GFP), yellow fluorescent protein (YFP), and cyan fluorescent protein (CFP)], as well as fusion constructs of DNAPK to these same fluorescent proteins. In the second half of year 3, we overexpressed all these fusion constructs in VCaP prostate cancer cells through stable transfection approaches, with the goal of assessing the ERG-DNAPK interaction in response to radiation. As expected, the DNAPK-GFP fusion protein was expressed diffusely in the nuclear compartment at baseline, but localized to distinct foci upon radiation treatment (Figure 1, left panel). The ERG-GFP fusion protein was expressed diffusely in both the cytoplasmic and nuclear compartments at baseline, but did not change in location upon radiation (Figure 1 right panel). Even when the level of ERG expression was decreased (via clonal selection of cells with lower levels of expression), the ERG-GFP protein continued to be expressed diffusely throughout the cell. This level of diffuse expression was confirmed via immunofluorescence microscopy approaches using the ERG antibody in both wild-type prostate cancer cells and human prostate cancer samples (data not shown). Unfortunately, this level of homogenous diffuse expression, as well as its lack of change in localization with radiation treatment, prevented us from characterizing the order of recruitment in the ERG-DNAPK interaction. Fundamentally, ERG appears to be overexpressed at such a high level in ERG-positive prostate cancers, that it was difficult to assess for treatment-induced colocalization of ERG with DNAPK, as we had initially proposed. While this limits our ability to assess recruitment of ERG by DNAPK and vice versa, our completion of subtasks 5A and 5B did allow us to define the location of ERG and DNAPK in prostate cancer cells, as well as changes in this localization upon radiation treatment.

Figure 1:

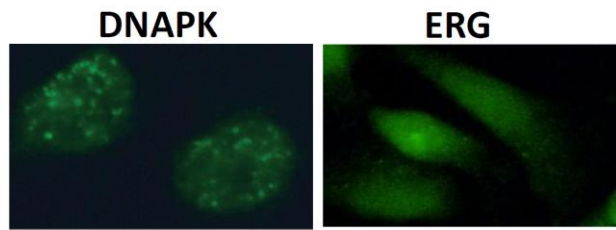


Fig 1: Overexpression of constructs fusing DNAPK to GFP (left) and ERG to GFP (right) was performed in VCaP prostate cancer cells. At baseline, DNAPK was located diffusely in the nuclear compartment, but upon radiation treatment, formed distinct foci in the nuclear component (left). At baseline, ERG was located diffusely in both the nuclear and cytoplasmic compartments, and this localization did not change upon radiation (right). Similar findings were obtained using DNAPK and ERG fused to other fluorescent proteins (YFP and CFP).

While subtasks #5A and #5B were expected to be completed over the final two years of the grant (years 4 and 5) on my initial statement of work, I moved up these subtasks to year #3 after experiencing some unanticipated delays in other subtasks. Specifically, I have experienced some unexpected delays in Specific Aim 3, which was to evaluate whether ETS gene fusion status is a predictive biomarker of resistance to radiation therapy in prostate cancer patients treated with external beam radiation. Specific Aim 3 was comprised of two tasks: Task #6 was to determine ETS gene fusion status in prostate cancer specimens from patients treated with radiation, and Task #7 was to determine the association between fusion status and clinical outcomes. My initial plan was to use a previously assembled institutional prostate cancer tissue set comprised of 281 specimens from men treated with radiation for prostate cancer. However, while trying to perform a separate (unrelated to this grant) biomarker study on these specimens, my pathologist (Rohit Mehra) and I realized that, unfortunately, the majority of these specimens are exhausted, from the perspective of cancer-containing tissue. More specifically, while the core biopsies from these specimens still exist, most of the cancer-containing tissue in these core biopsies had been exhausted in previous biomarker studies. Unfortunately, we could not determine this until we actually cut into each biopsy specimen and analyzed the biopsy slices under the microscope.

In order to continue with Specific Aim 3, I obtained another source of tumor specimens from men treated with radiation therapy for prostate cancer. I applied for tissue specimens from the phase III RTOG 96-01, which was run by the Radiation Therapy Oncology Group (RTOG), a national clinical trials cooperative group. RTOG 96-01 randomized 771 patients with PSA recurrences following prostatectomy to radiation therapy alone versus radiation therapy combined with androgen deprivation therapy. Of the 771 patients enrolled on the trial, prostate cancer samples are available for 88% of them, and the current median follow-up for these patients is 9.9 years. This cohort is exceptional in that it represents a large patient population with aggressive prostate cancer treated with radiation, with long-term clinical outcomes. The addition of androgen deprivation therapy to radiation resulted in a 17% decrease in recurrence rates (from 60% for the radiation alone group down to 43% for the combination therapy group), as well as a 6% decrease in the distant metastasis rate (from 13% in the radiation alone group to 7% in the combination therapy group). Both of these decreases were statistically significant, as reported in abstract form at the 2010 ASTRO Annual Meeting¹.

After a lengthy application and review process, the RTOG steering committee approved my request to assess ETS fusion status in these tissues. In addition, as the RTOG 96-01 tissues have previously been assembled into a tissue microarray, obtaining RTOG approval for my proposed project allowed me to accomplish Subtask 6B (assembly of prostate cancer specimens into a tissue microarray). However, over the past year, the RTOG changed its tissue request policies, such that all approval of tissue requests by the RTOG Steering Committee needs to be confirmed by the CTEP branch of the National Institutes of Health. The CTEP review of my tissue request is thus ongoing, but this additional level of review has delayed my ability to perform my proposed biomarker studies. After obtaining CTEP approval, I will then submit the appropriate documentation to the DOD IRB office (and obtain DOD approval) prior to proceeding with the proposed project.

In addition to the delays with the proposed biomarker studies, I also experienced a second delay in my proposed animal studies. Over the past year, I have been trying to secure in vivo quantities of a clinical grade DNAPK inhibitor (CC115) for my proposed in vivo studies. While my initial proposal included use of a preclinical DNAPK inhibitor (NU7026), I had wanted to substitute the clinical-grade inhibitor (CC115) instead in my experiments, as this would increase the translational relevance of any findings. However, it appears that the company which makes CC115 is not willing to provide their drug for studies combining this agent with radiation; thus, in the upcoming year, I will proceed with the in vivo experiments with NU7026 as initially proposed. I have already obtained animal use approval from my institution for these experiments, and will be submitting my animal use application to the DOD for their approval shortly.

Thus, to summarize, Year 3 of my grant period has been marked with the identification of potential barriers to my original intended plan--specifically, with the recognition that a clinical-grade DNAPK inhibitor (CC115) is not accessible for my *in vivo* studies, and with the realization that the tissue samples that I had planned on using for human biomarker studies was exhausted in terms of the cancer-containing component. However, I have developed solutions to overcome these barriers (using a preclinical DNAPK inhibitor instead of CC115, and obtaining access to a better tissue bank than the one I originally specified). Despite these barriers, I have accomplished 3 subtasks during year #3 of this grant, and over the first 3 years of this grant, I have completed 15 out of 20 proposed subtasks, which puts me ahead of the schedule outlined in my initial statement of work (12 subtasks to be completed over the first 3 years). Overall, the first three years of this grant have been quite successful.

Research achievements: Milestones

In the original Statement of Work, 11 milestones were identified, and targeted over the 5 year course of this grant. In year 1, I was able to complete Milestones #2, #4, and #5, for a total of 3 out of 11 milestones reached. In year 2, I was able to complete Milestones #1, #7, and #8, for an additional 3 milestones. In year 3, based on the work above, I was able to achieve Milestone #6 (data from Specific Aim #2 (from years #1 and 2) was included in a publication in *Cancer Discovery*) and Milestone #9 (tissue microarray). Thus, in total, I have reached a total of 8 out of 11 milestones during the first 3 years of this proposal, which is ahead of schedule (the original target on the Statement of Work was 6 milestones achieved by the end of year #3).

Training achievements

In my original grant application, I highlighted a series of training program activities which I hoped would contribute substantially to my scientific development. Over the past year, as proposed, I have continued to attend a number of basic science seminars, hosted by the Departments Medicine, and Molecular and Cellular Biology, which have broadened by scientific knowledge within my field. I have also regularly attended Gene Fusion and Cancer Biology Research Meetings, run by my mentor Arul Chinnaiyan, as well as the Pathology and Radiation Oncology Research Seminars, run by the two departments with which I am affiliated. Additionally, I have renewed my "Training in the Responsible Conduct of Research" certification, and presented at the national meetings noted above in the milestones section. Finally, I have met regularly with my mentors, Drs. Arul Chinnaiyan, Ted Lawrence, and Tom Carey, as planned in my original proposal.

Career achievements

The overall goal of my DOD Mentored Physician Research Training Award was to help me develop a career as a physician scientist committed to prostate cancer research. The first two years of this award have really helped launch my career in this regard. Because of my need to obtain tissue specimens to fulfill Aim 3 of this grant, I approached the Radiation Therapy Oncology Group (RTOG), and began regularly attending their Genitourinary Cancer Translational Research Committee meetings. Because of my increasing involvement with this group, I was appointed as chair of this committee. As chair of this committee, my role is to help direct RTOG-based prostate cancer research on a national level. This role has resulted in national recognition, as I was asked to present my research from this DOD grant in the 2011 AACR Prostate Cancer conference. Similarly, I moderated one of the 3 sessions at the ASCO GU conference this past year (my session was focused on translational research in prostate cancer). Over the past year, I have also served as a grant reviewer for the NIH Cancer Biomarker Study Section and a DOD study section (the Experimental Therapeutics section for Postdoctoral grants and Physician Research Training Awards). My DOD-sponsored project has led to the preliminary data necessary for several grants that I have received over the past two years, including a Celgene Translational Award (\$500,000 over 2 years) and a Prostate Cancer Challenge Award (\$1,000,000 split among 4 co-Principal Investigators over 2 years). In addition, I just received notice

that I was funded for three additional grants (one on which I am PI, the other two on which I am a co-PI). One of these grants, from the Fund for Cancer Research (\$75,000 for 1 year), was based directly on extending the work initiated in Aim 1 of this DOD PCRP grant. The other two grants (both Prostate Cancer Foundation Challenge Awards) are not directly related to the work included in this DOD PCRP grant, but do focus on different aspects of prostate cancer. In addition, over the past year, I have had three manuscripts accepted for publication or in press based on work from this proposal (detailed in the reportable outcomes section below). In addition to these 3 manuscripts, I have published 12 additional manuscripts over the past year, including 7 on which I am senior, co-senior, or first author. I would like to thank the DOD for making all of this possible for me.

Key Research Accomplishments:

The key research accomplishments from the third year of this grant proposal include the following:

- Generation of 3 different sets of VCaP prostate cancer cell lines expressing fluorescent fusion proteins of ERG and DNAPK (fused to GFP, YFP, and CFP)
- Characterization of the localization (and changes in localization) of ERG and DNAPK in response to radiation
- Approval of my application for prostate cancer tissues from the national phase III RTOG 96-01 trial, for use in my proposed biomarker studies
- Obtaining tentative access to a microarray compiled from RTOG 96-01 tissue

These accomplishments add to the findings from the first two years of the grant proposal, which showed that:

- ERG overexpression in prostate cancer cell lines confers radiation resistance
- This ERG-associated radiation resistance is mediated by increased efficiency of DNA repair in response to radiation
- ERG interacts with the repair protein DNAPK in a DNA-independent manner, at its tyrosine 373 site
- DNAPK knockdown or inhibition preferentially radiosensitizes ERG-positive vs ERG-negative cells, and can reverse ERG-mediated radiation resistance

Reportable Outcomes:

The third year of work from this grant proposal has resulted in the following reportable outcomes:

- 1) A manuscript, based on data from Aim 2 of this grant, published in the journal *Cancer Discovery*²
- 2) A publication on ETS gene fusions in prostate cancer, published in the journal *Curr Drug Targets*³
- 3) An invited review (on targeting ETS gene fusions), which has been accepted, pending minor revisions, to the journal *Clinical Cancer Research*⁴
- 4) A funded grant from the Fund For Cancer Research, entitled "Investigating ETS Gene Fusions as Predictive Biomarkers of Radiation Resistance and Targets for Radiosensitization" (\$75,000 over 1 year)

These outcomes add to the following reportable outcomes from the two year of the grant:

- 5) Publication of work from Task #4 in a *Cancer Cell* manuscript³, co-published with my mentor and primary collaborator, Dr. Arul Chinnaiyan⁵
- 6) Oral presentation on work from Task #4, at the 2010 American Society of Therapeutic Radiology and Oncology Annual Meeting⁶
- 7) Poster discussion presenting work from Tasks #1 and #3, at the 2011 American Society of Clinical Oncology Annual Meeting⁷
- 8) Invited oral presentation on work from Tasks #1 and #3, at the 2011 Prostate Cancer Foundation Annual Meeting
- 9) A funded Young Investigator Award from the Prostate Cancer Foundation (\$225,000 over 3 years), entitled "*Cooperativity between TMPRSS2:ERG Gene Fusions and PTEN Genomic Deletions in the Radiation Resistance of Prostate Cancer*", from January 2011 to January 2014
- 10) Oral presentation of work from this grant proposal, at the 2012 AACR Prostate Cancer Conference
- 11) A funded Challenge Grant from the Prostate Cancer Foundation (\$1,000,000 over 2 years, split among 4 co-principal investigators, of which I am one), entitled "Interrogating DNA repair aberrations in advanced prostate cancer"
- 12) A funded Translational Award from the pharmaceutical company Celgene (\$500,000 over 2 years, on which I am PI), entitled "CC115 as a therapeutic approach for metastatic Ewing's sarcoma or prostate cancer"

Conclusion:

This Annual Report summarizes the third-year accomplishments associated with the Department of Defense Physician Research Training Award (W81XWH-10-1-0582), awarded to Felix Feng, M.D. Overall, the third year of this grant period has been successful, and has resulted in three manuscripts published or in press (*Cancer Discovery*, *Current Drug Targets*, and *Clinical Cancer Research*) and one funded grant. These

accomplishments add to those achieved during the first two year of this grant, including one publication (*Cancer Cell*), three funded grants, and four presentations. In total, the first three years of this grant have resulted in four subsequent funded grants, four publications, four presentations, and a national leadership position. In addition, I have completed 15 out of the 20 subtasks proposed for this 5 year grant, and am ahead of the schedule proposed in the initial Statement of Work, despite having to overcome unexpected barriers to success in both the proposed xenograft and biomarker work. Additionally, I have met the training achievements specified in my original grant.

The research proposed in this training grant represents an important area within the field of prostate cancer research. Because ETS gene fusions are thought to be driver alterations in over half of all prostate cancers, understanding the mechanistic and potential clinical implications of these gene fusions has significant ramifications, particularly in the context of radiation therapy, which represents one of the primary treatment modalities for localized prostate cancer. Our findings are that ERG confers radiation resistance in preclinical models of prostate cancer and that this radiation resistance can be reversed with DNAPK inhibition. These findings suggest that DNA-PK inhibition should be explored as a clinical strategy for radiosensitizing prostate cancers. In addition, our accomplishments have now paved the way for us to continue with the necessary xenograft and human biomarker studies necessary to translate this work to the clinic.

I would like to thank the DOD review committee for providing me this grant to accomplish the proposed research.

References:

- 1) Shipley W, Hunt D, Lukka H, et al: Report of RTOG 9601: a Phase III Trial in Prostate Cancer: Anti-androgen Therapy (AAT) with Bicalutamide during and after Radiation Therapy (RT) Improves Freedom from Progression and Reduces the Incidence of Metastatic Disease in Patients following Radical Prostatectomy (RP) with pT2-3, N0 Disease, and Elevated PSA Levels. *International Journal of Radiation Oncology, Biology, Physics*. 78:S27, 2010
- 2) Goodwin JF, Schiewer M, Dean JL, Schrecengost RS, Han S, Den RB, Dicker AP, Feng FY, and Knudsen KE. A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discovery*. Accepted and in press.
- 3) White NM, Feng FY, Maher CA. Recurrent rearrangements in prostate cancer: causes and therapeutic potential. *Curr Drug Targets*. 14(4):450-9, 2013.
- 4) Feng FY, Brenner JC, Hussain MA, and Chinnaiyan AM. Molecular Pathways: Targeting ETS Gene Fusions. *Clinical Cancer Research* (accepted pending minor revisions)
- 5) Brenner JC, Ateeq B, Li Y, et al: Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer. *Cancer Cell* 19:664-78, 2011
- 6) Sabolch A, Brenner JC, Ateeq B, et al: Targeted Radiosensitization of ETS Gene Fusion- Positive Prostate Cancer. *International Journal of Radiation Oncology, Biology, and Physics* 78:S113, 2010
- 7) Feng FY, Han S, Brenner C, et al: PARP inhibition reverses radiation resistance conferred by ETS fusions in prostate cancer. *J Clin Oncol* 29:4545, 2011

Appendices:

None