Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** University of Pittsburgh- of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Margaret C. McDonald, Ph.D.

4. **Grant Contact Person's Telephone Number:** 412-383-7474

5. **Grant SAP Number:** 4100047655

6. **Project Number and Title of Research Project:** 06 – Estrogen Receptor Genetics in Breast Cancer

7. **Start and End Date of Research Project:** 3/30/2011 – 12/31/2012

8. **Name of Principal Investigator for the Research Project:** Adrian Lee, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 2,041,532.23

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Lee	Professor, PI	0.6% Yr 1; 16.7% Yr 2; 10% Yr 3	$ 73,676
Flint	Research Assistant Professor	34.6% Yr 2; 37.5% Yr 3	$ 69,208
Huang	Research Assistant Professor	14.6% Yr 2; 17.2% Yr 3	$ 31,719
Neumann	Associate Professor	25% Yr 3	$ 39,125
Wan	Associate Professor	11.7% Yr 2; 10% Yr 3	$ 34,311
Zhang	Assistant Professor	8.4% Yr 3	$ 10,350
Chan	Visiting Research Associate	29.2% Yr 2; 25% Yr 3	$ 40,157
Chandran	Postdoc Assignment	41.7% Yr 3	$ 25,424
Farabaugh	Postdoc Assignment	29.2% Yr 2; 25% Yr 3	$ 30,668
Boone	Postdoc Associate	88.3% Yr 2; 18.7% Yr 3	$ 56,678
Turaga	Temp Staff	25% Yr 2; 25% Yr 3	$ 9,615
Oesterreich	Professor	3.6% Yr 1; 23.3% Yr 2; 13.5% Yr 3	$107,084
Chen	Senior Research Specialist	16.7% Yr 2; 16.7% Yr 3	$ 22,745
Palmieri	Research Specialist	8.3% Yr 2; 50% Yr 3	$ 22,461
Ramgopal	Research Specialist	45.8% Yr 2; 4.5% Yr 3	$ 16,853
Sallit	Research Specialist	100% Yr 2; 8.3% Yr 3	$ 40,260
Behrini	Student Employee	50% Yr 2	$ 5,871
Anderson	Graduate Student	1.3% Yr 2; 7.6% Yr 3	$ 2,183

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
An oncogenic role for BRIP1 in breast cancer	☐NIH X Other federal (specify:_DOD__) ☐ Nonfederal source (specify:_)	12/2012	$561,915	$ pending

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

- Submit proposal to study estrogen receptor (ER) regulation in invasive lobular cancer (ILC) (NCI, R01, June 2013)
- Submit proposal to study epigenetic regulation of ER-regulated pathways in breast cancer (NCI, September 2013)
- Submit proposal to study role of mutation in ER binding sites in aromatase inhibitor (AI) resistance (NCI, R21, June or September 2013)

12. Future of Research Project. What are the future plans for this research project?

In this project we have investigated the genetics of the estrogen receptor (ER) in breast cancer. The ER is well recognized as one of the major drivers of breast cancer, and is currently targeted by antiestrogens for both prevention and treatment. However, the exact reason why the ER is such an important factor in breast cancer remains unclear, and its exact mechanism of action remains poorly understood. It is widely believed that a better understanding of ER action would lead to better methods to prevent and alleviate resistance to this excellent therapy and thus, breast cancer recurrence and death.

In this project we found that the ER gene (ESR1) is not somatically mutated in breast cancer. This is a fundamentally important observation, which, although negative, is important to the field. Despite this, we have found interesting differences in regulation of ER protein levels and are studying this observation in depth as these differences may contribute to endocrine therapy resistance. We expect that understanding ER degradation may provide novel therapeutic options for breast cancer.

We examined the hypothesis that SNPs (single nucleotide polymorphisms) in ER binding sites may affect a woman's response to both estrogen and antiestrogens. Through various bioinformatic approaches we identified novel SNPs in ER binding sites, and we plan to test how these changes affect hormone receptor activity and response.

Finally, we have continued to examine the intriguing role of ER in mediating genomic translocations. We identified a gene, PLAC1, which is normally expressed only in the placenta, that is estrogen regulated in breast cancer cell lines, and we tied this regulation to a genomic translocation of an ER binding site upstream of the gene. Others have shown similar results for androgen receptor (AR) in prostate cancer. Examining this phenomenon on a genome-wide scale, we find enrichment for ER binding sites at locations of genomic translocations. In the future we intend to study the mechanism whereby ER mediates genomic change and the importance of this mechanism on breast cancer initiation and progression.

In summary, the project has revealed new insights into the genetics of breast cancer and has set a foundation for future work to investigate how host and tumor genetics affect ER, response to hormone, and breast cancer.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes____X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    With this project we were able to initiate collaboration with key personnel which we hope will be long-lasting and ultimately result in novel insights that can only come when different research fields come together. For example, we worked closely with many bioinformaticians to analyze genomic data, and we hope that this in silico work will guide our wet lab research and validation.

16. **Collaboration, business and community involvement.**

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X_____ No_____

If yes, please describe the collaborations:

Aleks Milosavljevic, Baylor College of Medicine, Houston, TX
Todd Skaar, Indiana University School of Medicine, Indianapolis, IN

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a

performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Progress:

Specific Aim 1: Identify and characterize single nucleotide polymorphisms (SNPs) and mutations in the estrogen receptor (ER) in human breast cancer

1.1) Examine the presence of ER SNPs and mutations identified in human breast cancers in a large panel of ER-positive breast cancer cell lines

The estrogen receptor alpha (ESR1) is a critical driver of breast tumorigenesis and, as such, has been a target of therapy for many years. Early reports using Sanger sequencing and conformational assays suggested that there were few, if any, mutations in ER-positive tumors, although a few somatic mutations have recently been described in tumors and cell lines. We set out to validate the authenticity of these reported somatic mutations by analyzing ER DNA sequence variants (DSVs) in 66 ER+ breast tumors and 39 breast cancer cell lines. Genomic DNA from primary breast tumors, part of the Baylor College of Medicine Breast Tumor DNA Bank, previously described in detail [1], was utilized. Briefly, tumors with an ER content of at least 3 fmol/mg protein were crushed, and DNA was isolated by using Puregene DNA Purification Kit (Qiagen). In addition, DNA was used from a large panel of breast cancer cell lines from the NCI Integrative Cancer Biology Program (ICBP) and ATCC 43 breast cancer cell line kit (ICBP43).

We used a combined approach of target capture sequencing of ESR1 and subsequent testing for novel and previously identified DSVs using orthogonal mass spectrometry-based sequencing. Selective capturing of ER exons using compartmentalized, microfluidic biochips was performed as previously described [2, 3]. Exon sequences of ESR1 were downloaded from NCBI and 50mer probes were tiled across the exon targets of the full region. Eluted samples were subjected to 10 cycles of PCR, purified by Qiagen MinElute PCR purification, quantified using the Nanodrop 3300 instrument, and sequenced using SOliD.

Sequences were mapped using BFAST and reference genome hg18. Monoclonal reads were removed using in-house scripts. SNPs were called using: SAMTools, SNVMix, and VarScan . SNPs called by at least two SNP callers were reported. The SNP calls were compared to the dbSNP 130 and the COSMIC variation databases. The SNPs/DSVs were further filtered by

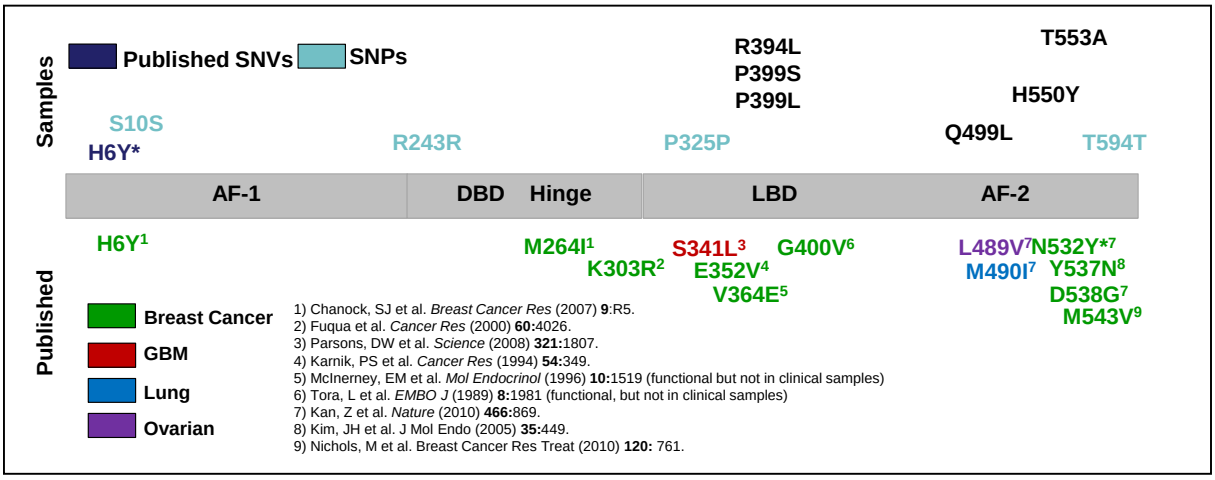
imposing at least two reads supporting the variant allele and requesting that the variant allele account for at least 20 % of the total number at reads, and requiring evidence for the variants in both strands. We identified 12 DSVs in ESR1 exons, five of which were previously described SNPs (rs2077647, rs746432, rs4986934, rs1801132, rs2228480). One DSV has previously been described as a mutation (H6Y), and six are potential novel DSVs (Table 1).

Table 1: Identification of DSVs in ESR1 coding region by capture-sequencing

		Nucleotide	**Amino acid**	**dbSNP**	**Reccurence**
1	152170756	**C16T**	**H6Y**	Not_dbSNP	1
2	*152170770*	***T30C***	***S10S***	*rs2077647*	*27*
3	*152171001*	***G261C***	***A87A***	*rs746432*	*8*
4	*152243568*	***T729C***	***R243R***	*rs4986934*	*16*
5	*152307215*	***G975C***	***P325P***	*rs1801132*	*12*
6	152374568	**G1181T**	**R394L**	Not_dbSNP	1
7	152374582	**C1195T**	**P399S**	Not_dbSNP	3
8	152374583	**C1196T**	**P399L**	Not_dbSNP	1
9	152457339	**A1496T**	**Q499L**	Not_dbSNP	1
10	152461654	**C1648T**	**H550Y**	Not_dbSNP	1
11	152461663	**A1657G**	**T553A**	Not_dbSNP	1
12	*152461788*	***G1782A***	***T594T***	*rs2228480*	*2*

For sequence confirmation we used a Maldi-Tof mass spectrometry from Sequenom, as previously described [6]. This technology has been shown to work with small amounts of degraded DNAs, its multiplexing capacity makes it highly efficient, and a variety of studies have demonstrated that the sensitivity of mass spectrometric methods exceeds that of traditional Sanger sequencing and is highly concordant with Sanger sequencing, Pyrosequencing, and allele-specific PCR [7-9]. This sequence analysis was conducted in collaboration with the National Surgical Adjuvant Breast and Bowed Project (NSABP) in Pittsburgh (Patrick Gavin). We developed mass-spectrometry assays, including controls, for these DSVs, and also included 10 DSVs that had previously been reported as somatic mutations in breast (and other) cancers (Fig 1).

Fig 1: **DSVs tested through mass-spec approach (Sequenom).**



Applying a Sequenom®-based analysis to breast tumors and cell lines, we were able to confirm previously reported SNPs and one previously reported mutation (H6Y). This mutation was found in one human breast tumor and one cell line. None of the other reported somatic mutations could be confirmed in tumors or cell lines. This result clearly indicates that ESR1 mutations are very rare in primary human breast tumors. This study is the largest ER sequencing study done to date, and, although negative, provides very critical information. A poster summarizing these data was presented at the San Antonio Breast Cancer Symposium (12/2012, Oesterreich et. al., "Lack of Frequent ER Mutations in Primary Breast Cancer"), and we are currently preparing the manuscript for submission.

1.2) Determine the biological importance of SNPs and mutations from Aim 1.1

1.2.1) Functional analysis of the ESR1 H6Y mutation

Given the identification of the ESR1H6Y mutation, we set out to identify the biological importance of this DSV. This was accomplished through ERE-tk-luciferase reporter assays (Fig 2). We introduced the H6Y (C16T) change into an HA-tagged ESR1 construct using site-directed mutagenesis (Quick Change® Site-Directed Mutagenesis Kit from Stratagene, La Jolla, CA). After confirming the correct sequence of the ESR1 coding region, effects on ESR1 transcriptional activity were determined in transient reporter assays using ERE-Tk-Luc. To determine potential effects on cell cycle, we measured the proportion of cells in S-phase through the incorporation of BrdU and analysis of cell cycle distribution in the presence of ER wildtype or H6Y (data not shown). These functional assays on the ESR1H6Y DSV failed to identify increased or altered activity of the mutant. The lack of a selective phenotype and the infrequent occurrence of this DSV (1/60 tumors) do not support a major role for ESR1H6Y. As stated above, this analysis suggests that ESR1 mutations are a rare event in ER+ primary breast cancer.

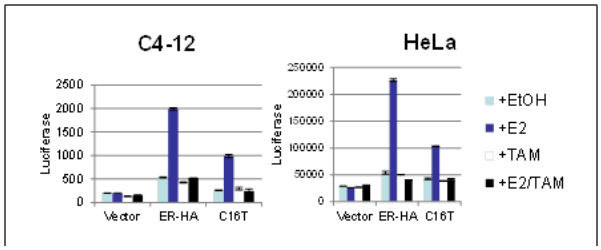


Fig 2: ESR1H6Y has similar transcriptional activity to wild-type ESR1. Cell lines (C4-12 and HeLa) were transiently transfected with ERE-Tk-reporter and with ESR1 wildtype (ER-HA), ESR1 mutation H6Y (C16T), or empty plasmid (vector) as a control. Cells were treated with vehicle (EtOH), 10-8M estradiol (E2), 10-6M 4-OH-tamoxifen (Tam), or a combination of 10-8M estradiol and 10-6M 4-OH-tamoxifen (E2/Tam). Luciferase values were determined using Luciferase reporter kit (Promega) and a Luminometer.

1.2.2) Functional analysis of ESR2 (ERβ) SNPs

In collaboration with investigators at Indiana University (IU), we also studied whether there were DSVs in the ESR2 gene. Specifically, a number of reports had previously shown that genetic variants in the ESR2 gene are associated with a variety of clinical phenotypes. However, very little was known about DSVs in the regulatory region of the gene or about the functional significance of ESR2 genetic variants. We used a bioinformatics approach to identify regions of the ESR2 promoter that are evolutionarily conserved across the genomes of several species. We re-sequenced 1.6 kb of the ESR2 gene, which included 0.8 kb of the promoter, 0.3 kb of exon ONE, and 0.5kb of the following intron. We identified five SNPs in the ESR2 promoter and one SNP in the intron. Three of the SNPs (rs8008187, rs3829768, rs35036378) were predicted to alter transcription factor binding sites in the ESR2 promoter; interestingly, the rs35036378 SNP was in the TATA box and was highly conserved across species. We performed functional studies similar to Aim 1.2.1 above. Briefly, a 410 bp sequence of the ESR2 promoter containing the TATA box (nucleotide 45761496 to 45761087 from accession # NT_026437) was cloned into the HindIII/XhoI site of the pGL3 Basic promoter-less vector (Promega). The plasmid with the variant TATA box was created using the Quick Change® Site-Directed Mutagenesis Kit, sequences were confirmed, and then reporter assays were performed in a number of cell lines. These assays showed that the rs35036378 SNP allele had approximately 50% less activity relative to the major allele. We conclude that the rs35036378 SNP appears to cause reduced promoter activity of the ESR2 gene. Since this SNP was only found in African American subjects, further studies are warranted to determine whether it could contribute to differential tumor development and progression. This paper has recently been published (Philips et al, *Hormones and Cancer*, 2012).

Given the negative data obtained from our studies conducted under Aim 1, i.e. the definitive lack of frequent mutations in the ESR1 gene, we have begun to study other potential causes of altered ER activity in breast cancer development and in resistance to endocrine therapy. Specifically, we are focusing on 2 questions: 1) Does altered ER protein turnover result in changes to endocrine treatment response? 2) Does ER engage in “inappropriate” interactions with co-regulatory proteins, resulting in increased proliferation and loss of hormone dependence of the tumor cells?

For the first question, we have begun to analyze how ER undergoes turnover (via the proteasome) and whether altered degradation may be responsible for resistance to hormone therapy. We examined the mechanism whereby Fulvestrant (ICI 184720), a selective estrogen down-regulator (SERD), causes degradation of ER. Using site-directed mutagenesis of ER similar to that described above, we found that Fulvestrant-mediated degradation requires a central domain of ER that contains the nuclear localization sequence. We have subsequently identified two lysine residues that are likely sumoylated and contribute to the ability of Fulvestrant to cause ER to be degraded. This work has been written up and is about to be submitted to a journal for publication. Interestingly, while studying ER degradation in a panel of breast cancer cell lines, we noticed that invasive lobular cancer (ILC) cell lines express very high levels of ER protein compared to invasive ductal cancer (IDC) cell lines, whereas there are no differences at the RNA levels (data not shown). We subsequently found that these cells lines are inherently resistant to antiestrogen, and preliminary data suggest that the ER in these cell lines is also resistant to hormone-mediated degradation. We have recently published a review summarizing data on hormone response in ILC (Sikora et al, *Steroids* 2012) and we are continuing an in-depth analysis of this phenomenon.

For the second question, we have begun to study the interaction between ER and repressive histone modifying enzymes. Briefly, we performed a screen for epigenetic changes (DNA promoter methylation) in endocrine resistant cell lines (C4-12 and LTED). In this unbiased screen we found that the estrogen-repressed gene HOXC10 was hypermethylated in endocrine resistant cells, and consistent with this finding, HOXC10 mRNA and protein were down-regulated in endocrine resistant cell lines and human tumors. Intriguingly, we observed that there was a step-wise epigenetic reprogramming of the HOXC10 promoter during the process of loss of hormone response, including an ER-EZH2 interaction, and bivalent chromatin marks, followed by DNA methylation. These studies have been submitted for publication, and are currently under review (*Science Translational Medicine*). We will now test whether inhibition of EZH2 will prevent endocrine resistance, at least in part through the prevention of epigenetic reprogramming of genes like HOXC10.

Specific Aim 2: Examine genome-wide binding of ER in breast cancer cell lines and identify SNPs and/or mutations in ER binding sites that affect hormone response

2.1) Perform ER-chromatin immunoprecipitation (ER-ChIP) and sequencing to identify genetic variants in ER binding sites in ER+ breast cancer cell lines, and correlate binding with transcriptional regulation using transcriptomic analysis

For the identification of DSV in ER binding sites we have pursued two approaches: 1) we have compiled lists of ER binding sites, through (a) performing our own ChIP-seq analysis in a number of breast cancer cell lines and other cell lines, which will provide us with insight into the role of ER in the metastatic process, and (b) by analyzing publicly available ChIP-seq data from breast cancer cell lines and tumors, and 2) we will use publicly available data sets with genome-wide sequencing information to identify DSVs in ER binding sites.

We performed ChIP-seq studies in a number of cell lines, including the estrogen-responsive osteoblastoma-like U2OS cells, the ER+ invasive ductal breast cancer cell line MCF-7, and the

invasive lobular cancer cell line MDA-MB-134VI. The studies in U2OS cells led to the identification of estrogen regulation of a prostaglandin transporter, which had not previously been implicated in estrogen-mediated tumor growth or metastasis. We have therefore begun a collaboration with Dr. Pawel Kalinksi (University of Pittsburgh Cancer Institute [UPCI], Pittsburgh, PA), an expert in the study of PGE2, to follow up on these studies. These studies are ongoing.

The ER binding sites in the ILC MDA-MB-134VI cells are currently being sequenced, and we expect to obtain this result within the next few weeks. The validity of our approach was confirmed by candidate gene PCR, using the ChIP DNA (Fig 3).

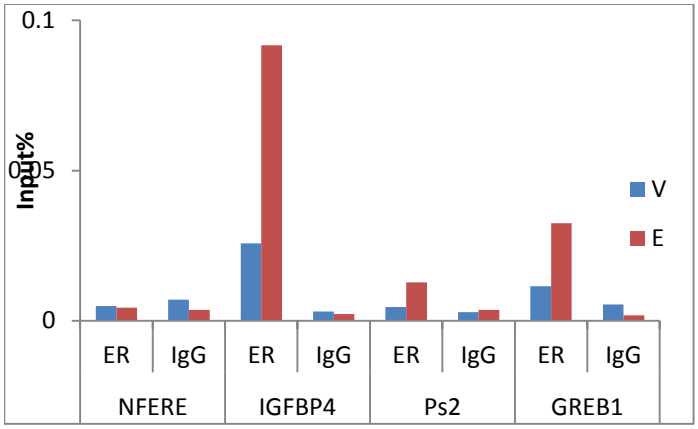


Fig 3: ChIP results in MDA-MB-134VI cells. Cells were incubated in 5% CSS, and treated with 10-8M E2 for 45 mins. ChIP assays and qPCR were performed, analyzing 3 candidate genes (IGFBP4, pS2, and GREB1). A non-functional ERE (NFERE) served as negative control.

We have already performed gene expression array studies in MDA-MB-134VI cells (-/+ estrogen, 6 hour and 24 hour time points) (these data were presented in a poster at the San Antonio Breast Cancer Symposium, 12/2012). The UPCI Biostatistics Facility is currently analyzing the gene expression results. We will combine the gene expression data set with the list of ER binding sites, with the expectation of identifying DSVs in ILC which alter hormone response in these cells. We also requested tumor samples from the University of Pittsburgh Health Sciences Tissue Bank, and we are currently isolating DNA and RNA from these samples, with the goal of confirming DSVs and correlating gene expression changes in clinical samples. Finally, we will be able to correlate these changes with response to endocrine therapy since the tumors we are using are extremely well-curated with respect to clinical information. Meanwhile, a number of groups have published ChIP-seq studies with ESR1. Therefore, in collaboration with Drs. Xinghua Lu and Panayiotis Benos (Center for Translational Bioinformatics, University of Pittsburgh), we downloaded publicly-available ChIP-seq datasets and identified DSVs in the datasets. As an example, for the MCF-7 cell line, we currently have access to nine ER ChIP-seq datasets. Focusing on the overlapping (i.e., high confidence) estrogen-induced ESR1 binding sites, we identified about 5,200 binding sites. Cross-referencing these sites with dbSNP and datasets from the 1000 Genomes Project showed that approximately 85% of these sites harbor known SNPs. The remaining 15% are either sequencing artifacts (which is unlikely, given the Genome Analysis Toolkit [GATK] workflow stringency criteria we applied to our analysis), or they are low frequency SNPs that have not yet been deposited into public databases, or they might actually be somatic mutations in ER binding sites.

To address this question, we decided to first decrease the number of unknown DSVs through the

development and application of bioinformatics models that predict the effect of the DSV on ER binding. We will modify JASPAR libraries, using ERE (and other ER-related recognition sites) PSSM (position specific scoring matrix). Briefly, we will run the code on the sequences with potential DSVs, identify EREs (with PSSM score > 80), mutate individual base pairs (WT, and mutant), rerun the code, and compare the results. In addition, a further analysis of these binding sites has also led to the discovery that there are fundamental differences in ER and cofactor recruitment between estrogen and repressed genes, which will need to be considered for functional analyses of the DSVs in binding sites. These novel observations have been submitted to *Genome Biology*, and are currently under review (Hatice Ulku Osmanbeyoglu, Roger Day, Steffi Oesterreich, Panayiotis V. Benos, Claudia Coronnello, Xinghua Lu. Estrogen represses gene expression through reconfiguring chromatin structures).

As expected, these studies have led to increased interaction and collaboration with our bioinformatics colleagues, especially Dr. Panayiotis Benos and Dr. Xinghua Lu (University of Pittsburgh). In collaboration, we have recently developed and tested an algorithm that identified DSVs in miRNA binding sites. The description of this model is in press (Coronello et al, A biophysical model of miRNA targeting identifies SNPs with potential role in bone biology. PLoS Computational Biology). We expect that our ongoing studies of DSVs in ER binding sites will contribute to the development of a bioinformatics model that we expect to publish.

In addition, we will determine whether there are DSVs in ER binding sites that are the result of somatic mutation in breast cancer. Over the last year there have been numerous reports of sequencing of human breast cancers, including studies from The Cancer Genome Atlas (TCGA) and others. These studies are available from dbGAP or TCGA directly. We have obtained approval for download of these data from dbGAP and have started to analyze the first dataset. We will focus on the ER binding regions studied above and catalog somatic mutations for wet lab validation.

2.2) Determine the effect of mutations and SNPs in ER binding sites on response of breast cancer cells to hormone and anti-hormone treatment

We have not yet begun this part of the Aim since we will need to identify the DSVs first.

Specific Aim 3: Determine the role of ER in orchestrating genomic instability and translocations

3.1) Test whether ER regulates levels of a gene (PLAC1) that shows translocation of ER binding sites upstream of its promoter region

ESR1 and other nuclear hormone receptors regulate gene transcription by bringing DNA enhancer elements (that are situated a long distance away) into proximity with promoters. This looping of DNA is a relatively unique action of nuclear hormone receptors but may represent a potential Achilles heel, as nuclear hormone receptor action in cancer cells with reduced DNA repair capacity may potentially increase chromosomal translocations. Consistent with this hypothesis, we identified a fetal placental gene, PLAC1, that is re-expressed at very high levels in MCF-7 breast cancer cells. In this cell line, PLAC1 is under estrogen regulation, which is not the case during placental development. We hypothesized that PLAC1 may have an ESR1

binding site translocation upstream of its promoter to confer *de novo* estrogen regulation. We performed paired-end sequencing of DNA and identified a genomic translocation of chromosome 6 upstream of the PLAC1 gene on the X chromosome, and this region of chromosome 6 harbors 3 ESR1 binding sites, validated by ESR1 ChIP (Figure 4).

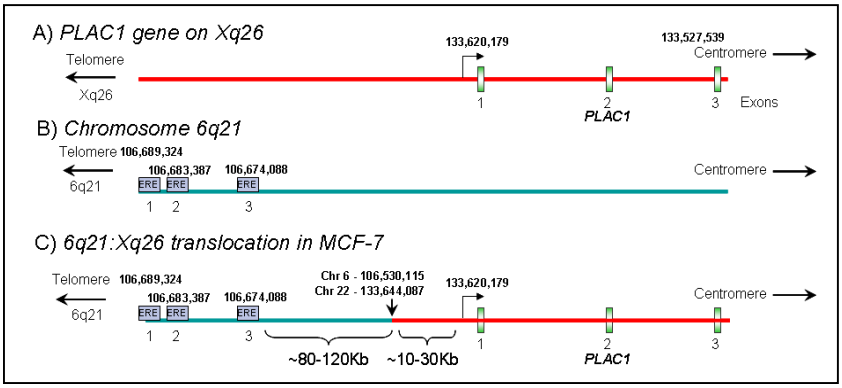


Figure 4: Schematic of the translocation involving PLAC1 in MCF-7 cells. A) PLAC1 gene on chromosome Xq26. B) Non-genic region of chromosome 6q21. C) Translocation occurring in the enhancer region of PLAC1. Note this finding brings three putative ER binding sites upstream of PLAC1. Please note that the genomic location of the translocation has only been mapped using fosmid end sequencing (one end was chr 6q21 and one end was chr Xq26). Given that the fosmids are approx 40Kb in size, this means that the translocation could be within 10-30Kb of the transcriptional start site. Further detailed mapping is currently underway to pinpoint the exact location of the translocation (and thus the distance of the ER binding sites). Note that the NRIP gene has a -144Kb distal enhancer that can interact directly with the NRIP promoter.

We found that PLAC1 is highly estrogen regulated in MCF-7 but not in five other ER+ breast cancer cell lines, each of which showed estrogen regulation of classical genes (PR [progesterone receptor] and PS2) (Fig 5). Further studies are investigating the biological role of estrogen regulation of PLAC1 in MCF-7 cells and the identification and validation of other ESR1-mediated translocations.

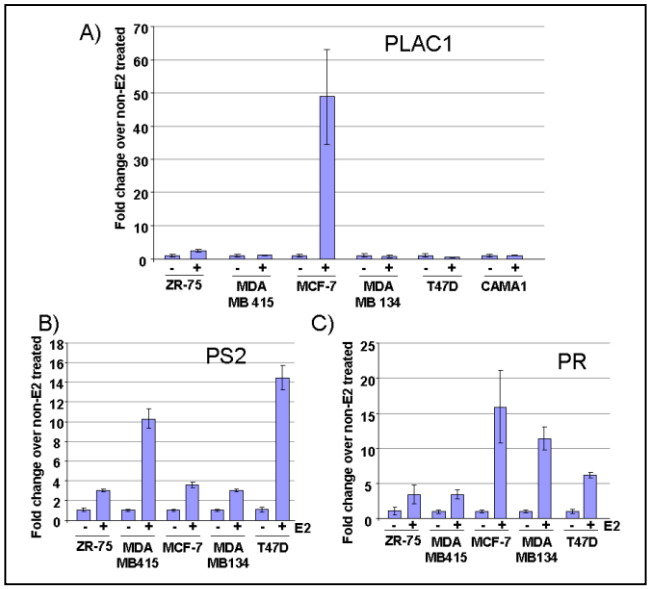


Figure 5: Cell line specific regulation of PLAC1. ER-positive breast cancer cell lines were cultured in 5% charcoal-stripped serum (CSS) and then stimulated with estradiol (1nM) for 24hrs. RNA was isolated and Q-RT-PCR performed for A) PLAC1, (B) PS2 and (C) PR. Bars represent the average of triplicate wells +/- S.E.M, and values represent fold control normalized to expression in the absence of estradiol.

3.2) Examine whether ER binding sites identified by ChIP-sequence (Aim 2) overlap with genomic breakpoints determined by paired-end sequencing

In collaboration with Drs. Aleksandar Milosavljevic and Christian Coarfa at Baylor College of Medicine, we next examined the association between ER and gene translocations in an unbiased genome-wide screen. We used two datasets for this analysis. First, we performed SOLiD 4kB mate-pair sequencing on three breast cancer cell lines (HCC1954, MDA-MB-231, and MDA-MB-361) and two human breast tumors. For the sequencing we received a clone coverage of 9.7-13.5x. The second was a public dataset imported from a landmark study in the U.K. of 24 human breast tumors. We identified putative genomic translocations and removed breakpoints that were found in the normal population (identified in the 1,000 Genomes Project).
We then examined statistical enrichment for epigenomic marks and transcription factor binding sites (such as ER binding site) in the dataset of genomic translocations (Figure 6). We used 259 epigenomic feature tracks from the following sources: 148 transcription factor binding tracks generated by the ENCODE project, 66 transcription factor binding tracks and 25 other epigenomic marks in breast cancer from the Cistrome database, and 20 normal epigenomic tracks from the Human Epigenome Atlas Release 2. We computed enrichment scores for each of the 259 epigenomic features for both germline and putatively somatic breakpoints using a 50,000 basepair radius around each breakpoint. A total of 107 (out of 259) features showed significantly different patterns of enrichment (Mann-Whitney-Wilcoxon test, p-value<0.05) between the two types of breakpoints. The enrichment scores for discriminating features were used to cluster the samples and the features (Figure 6).

Among the 74 features enriched around somatic breakpoints are ESR1 (8 datasets), FoxA1 (2 datasets), PGR, and SRC-3. These findings support our previous observation of ER-directed translocation at the PLAC1 promoter. We observed strong enrichment around somatic translocations for 49 transcription factors profiled by the ENCODE project, including GATA-1/2, c-Jun, CEBPB, FoxA1, ERα, and p300 ($p<0.02$). To examine possible interactions between ESR1 and other transcription factors for each of the ESR1 experiments, we identified somatic breakpoints within 50kb of an ESR1 binding site and then identified genes within 10kb of such breakpoints. A total of 40 genes were identified by this method in 3/21 cancer samples. Enrichment analysis of the 40 genes by GSEA indicated enrichment for ETS1 (p-value=3.23×10^{-4}) and p53 (p-value=3.71×10^{-4}) binding in promoter regions of these genes. The enrichment patterns suggest that transcription factors either individually or as part of larger complexes may promote genomic instability. A manuscript describing this work and the associated bioinformatic platform is in preparation for submission.

Figure 6. Hierarchical clustering of breakpoint sets based on enrichment for transcription factor binding and epigenomic features. Note the distinct patterns of enrichment for germline breakpoints (columns to the left) and putatively somatic breakpoints (columns to the right).

References:
1. Pathiraja, T.N., et al., Progesterone receptor isoform-specific promoter methylation: association of PRA promoter methylation with worse outcome in breast cancer patients. Clin Cancer Res, 2011. **17**(12): p. 4177-86.
2. Summerer, D., et al., Microarray-based multicycle-enrichment of genomic subsets for targeted next-generation sequencing. Genome Res, 2009. **19**(9): p. 1616-21.
3. Bau, S., et al., Targeted next-generation sequencing by capture of multiple genomic loci using low-volume microfluidic DNA arrays. Anal Bioanal Chem, 2009. **393**(1): p 171-5.
4. Baum, M., et al., Validation of a novel, fully integrated and flexible microarray benchtop facility for gene expression profiling. Nucleic Acids Res, 2003. **31**(23): p. e151.
5. Guimil, R., et al., Geniom technology--the benchtop array facility. Nucleosides Nucleotides Nucleic Acids, 2003. **22**(5-8): p. 1721-3.
6. Fumagalli, D., et al., A rapid, sensitive, reproducible and cost-effective method for mutation profiling of colon cancer and metastatic lymph nodes. BMC Cancer, 2010. **10**: p. 101.
7. Thomas, R.K., et al., High-throughput oncogene mutation profiling in human cancer. Nat Genet, 2007. **39**(3): p. 347-51.
8. Vivante, A., et al., High-throughput, sensitive and quantitative assay for the detection of BCR-ABL kinase domain mutations. Leukemia, 2007. **21**(6): p. 1318-21.
9. van Puijenbroek, M., et al., Mass spectrometry-based loss of heterozygosity analysis of single-nucleotide polymorphism loci in paraffin embedded tumors using the MassEXTEND assay: single-nucleotide polymorphism loss of heterozygosity analysis of the protein tyrosine phosphatase receptor type J in familial colorectal cancer. J Mol Diagn, 2005. **7**(5): p. 623-30.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?

_____Yes

__X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Functional characterization of a genetic polymorphism in the promoter of the ESR2 gene	Philip, Richter, Oesterreich, Rae, Flockhart, Reumal, Skaar	Hormones and Cancer	May 2011	☐Submitted ☐Accepted X Published
2. Novel modeling of combinatorial miRNA targeting identifies SNP with potential role in bone density	Coronello, Hartmaier, Arora, Huleihel, Pandit, Bais, Butterowrth, Kaminski, Stormo, Oesterreich, Benos	PloS Computational Biology	June 2012	☐Submitted ☐Accepted X Published
3. Invasive lobular carcinoma of the breast: Patient response to systemic endocrine therapy, and hormone response in model systems	Sikora, Jankowitz, Dabbs, Oesterreich	Steroids	Sept 2012	☐Submitted ☐Accepted X Published
4. HOXC10 loss in breast cancer – Epigenetic reprogramming of developmental genes in endocrine resistance	Pathiraja, Nayak, Xi, Jiang, Garee, Edwards, Lee, Shea, Santen, Gannon, Kangaspeska, Jelinek, Issa, Richer, Elias, McIlroy, Young, Normolle, Schiff, Li, and Oesterreich	Science Translational Medicine	December 2012	X Submitted ☐Accepted ☐ Published
5. Estrogen represses gene expression through reconfiguring chromatin structures	Hatice Ulku Osmanbeyoglu, Roger Day, Steffi Oesterreich, Panayiotis V. Benos, Claudia Coronnello, Xinghua Lu.	Genome Biology	December 2012	X Submitted ☐Accepted ☐ Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____X____ No_____

If yes, please describe your plans:

We are planning to submit within the next 1-2 months:

Oesterreich, Kitchens, Gavin, Wu, Riehle, Coarfa, Edwards, Schiff, Milosavljevic, Lee: Lack of Frequent Estrogen Receptor Mutation in Primary Breast Cancer.

We are also planning to submit manuscripts on work that is based on this project but not yet completed. We anticipate submitting at least three additional manuscripts on the following topics:

1) Identification of ER and SRC1 binding sites
2) Effect of DNA sequence variants in ER binding sites on hormone response
3) Analysis of ER mutation in recurrent breast tumor specimens
4) Changes in ESR1 promoter methylation in aging breast cancer
5) Differences in ER levels and activities between IDC and ILC
6) Enrichment for ER binding sites at location of genomic rearrangements in breast cancer
7) Identification of the ER nuclear localization sequence as critical for Faslodex-mediated degradation of ER.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

This project has not directly affected breast cancer diagnosis or prognosis, but it is anticipated that a better understanding of ER action will lead to changes in the response to endocrine therapy.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:
b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME **Adrian V. Lee**	POSITION TITLE **Professor**
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Kent, Canterbury, UK	B.Sc.	1986-1989	Biochemistry
ICRF, University of Surrey, Guildford, UK	Ph.D.	1990-1993	Breast Biology
UT Health Science Center at San Antonio	Postdoc	1993-1997	Breast Cancer

Positions and Honors

Positions

1997-1999 Instructor, Dept. of Medicine, University of Texas Health Science Center, San Antonio, TX

1999-2005 Assistant Professor, Breast Center, Dept. of Medicine, and Dept. of Mol. & Cellular Biology, Baylor College of Medicine, Houston, TX

2005-2010 Associate Professor, Breast Center, Dept. of Medicine, and Dept. of Mol. & Cellular Biology, Baylor College of Medicine, Houston, TX

2010-present Professor, Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA.

2010-present Director, Women's Cancer Research Center, University of Pittsburgh Cancer Institute, Pittsburgh, PA

Awards

1990 Imperial Cancer Research Fund, graduate scholarship

1998 New Faculty Start Up Award, Howard Hughes Medical Institute

1998 NCI/SPORE Career Development Award in Breast Cancer

2003 T.T. Chao Scholar Award

Honors

1998-2008 Member, Department of Defense study sections; Endocrinology III (1998), Endocrinology II (2000-2002), Pathobiology II (2005), Endocrinology I (2006), Integration Panel (2008)

1999-2002 Permanent Member, Tumor Cell Biology study section, Komen Foundation

1999-2010 Organizing Committee, San Antonio Breast Cancer Symposium

2000-2001 Scientific Advisor, Komen Research Foundation

2001 Member, Massachusetts Department of Public Health Breast Cancer study section

2003-2007 Permanent member, California Breast Cancer Research Program, Etiology and Prevention

2005-2010 Ad hoc member NIH study sections; SEP – Molecular Targets for Cancer Drug Discovery (2003), P01– Molecular Carcinogenesis (2005), Molecular Oncogenesis (MONC) (2005-2007), SEP ZRG1 EMNR-E (02) (2009), SEP ZCA1 PCRB-B (A1) S (2009), NCI intramural MTBL program review (2010),

2006 Ad hoc member, NCIC Program Project Review – IGF as a therapeutic target

2008 Susan G Komen for the Cure Pathobiology study section (PBY)

2010 Executive Committee, San Antonio Breast Cancer Symposium

2011 Chair, Susan G Komen for the Cure Postdoctoral Fellowships – Tumor Cell Biology

2005-present Section Editor (Viewpoints), Breast Cancer Research

2007-2012	Permanent member, NIH Molecular Oncogenesis study section (MONC)
2009-present	Editorial Board, Hormones and Cancer; Hormone Molecular Biology and Clinical Investigation
2010-present	Scientific Advisory Council, Susan G Komen for the Cure
2012	Ad hoc member, ZCA1 SRLB-C (J1) – SEP: Cancer Biology-2
2012	Chair, Susan G Komen for the Cure Career Catalyst Awards – Basic Science

Selected Peer-reviewed Publications

1. Creighton CJ, Casa A, Lazard Z, Huang S, Tsimelzon A, Hilsenbeck SG, Osborne CK, and **Lee AV**. Insulin-like growth factor I (IGF-I) activates gene transcription programs strongly associated with poor breast cancer prognosis. J Clin Oncol, 26:4078-85. 2008. PMID: 18757322. PMC2654368
2. Litzenburger BC, Kim H-J, Kuiatse I, Carboni JM, Attar RM, Gottardis MM, Fairchild CR, and **Lee AV**. BMS-536924 reverses IGF-IR-induced transformation of mammary epithelial cells and causes growth inhibition and polarization of MCF-7 cells. Clin Cancer Res 2009; 15: 226-37. PMID: 19118050. PMC2819349
3. Liu S, Umezu-Goto M, Murph M, Lu Y, Liu W, Zhang F, Cui X, Murrow G, Coombes K, Muller W, Hung MC, Perou CM, **Lee AV**, Fang X, Mills GB. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. Cancer Cell. 2009 2;15(6):539-50. PMID: 19477432
4. Migliaccio I, Osborne, CK, Gutierrez C, Allred DC, Mohsin S, Hilsenbeck SG, **Lee AV**. Nuclear IRS-1 predicts tamoxifen response in early breast cancer. Breast Cancer Research and Treatment, 2010;123:651-60. PMID: 19924529. PMC2891842
5. PMID: 19924529Dearth RK, Delgado DA, Hiney JK, Pathiraja T, Oesterreich S, Medina D, Dees WL, **Lee AV.** Parity-induced decrease in systemic growth hormone alters mammary gland signaling: a potential role in pregnancy protection from breast cancer. Cancer Prev Res. 2010 Mar;3(3):312-21 PMID: 20145191. PMC2833223
6. Gualberto A, Dolled-Filhart M, Gustavson M, Christiansen JJ, Wang YF, Hixon ML, Reynolds JM, McDonald S, Ang A, Rimm DL, Langer C, Blakely J, Garland LL, Paz-Ares L, Karp DD, **Lee AV**. Molecular Analysis of Non-Small Cell Lung Cancer (NSCLC) Identifies Subsets with Different Sensitivity to Insulin like Growth Factor I Receptor (IGF-IR) Inhibition. Clin Cancer Res. 2010;16:4654-65. PMID: 20670944. PMC2952544
7. Litzenburger BC, Creighton CJ, Tsimelzon A, Chan BT, Hilsenbeck SG, Wang T, Carboni JM, Gottardis MM, Huang F, Chang JC, Lewis MT, Rimawi MF, **Lee AV**. High IGF-IR activity in triple-negative breast cancer cell lines and tumorgrafts correlates with sensitivity to anti-IGF-IR therapy. Clin Cancer Res. 2011 Apr 15;17(8):2314-27.
8. Casa AJ, Potter AS, Malik S, Lazard Z, Kuiatse I, Kim HT, Tsimelzon A, Creighton CJ, Hilsenbeck SG, Brown PH, Oesterreich S, **Lee AV**. Estrogen and insulin-like growth factor-I (IGF-I) independently down-regulate critical repressors of breast cancer growth. Breast Cancer Res Treat. 2011 May 4.
9. Dearth RK, Kuiatse I, Wang YF, Liao L, Hilsenbeck SG, Brown PH, Xu J, **Lee AV**. A moderate elevation of circulating levels of IGF-I does not alter ErbB2 induced mammary tumorigenesis. BMC Cancer. 2011 Aug 25;11(1):377. PMID: 21867536
10. Hampton OA, Miller CA, Koriabine M, Li J, Den Hollander P, Carbone L, Nefedov M, Ten Hallers BF, **Lee AV**, De Jong PJ, Milosavljevic. A Long-range massively parallel mate pair sequencing detects distinct mutations and similar patterns of structural mutability in two breast cancer cell lines. Cancer Genet. 2011 Aug;204(8):447-57.
11. Potter AS, Casa AJ, **Lee AV.** Forkhead box A1 (FOXA1) is a key mediator of insulin-like growth factor I (IGF-I) activity. J Cell Biochem. 2012 Jan;113(1):110-21