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TITLE: Proteogenomic Approaches for Finding Therapeutic Vulnerabilities in Metastatic Breast Tumors Expressing Transcriptionally Active ESR1 Fusions

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14. ABSTRACT Estrogen receptor- α positive (ER+) breast cancer is often initially responsive to endocrine therapy (ET), but many patients succumb to metastatic ET-resistant disease. This project focuses on drivers of ET resistance in the form of transcriptionally active, in-frame <i>ESR1</i> gene fusions, which encode chimeric proteins completely refractory to all ET due to the lack of the ligand binding domain of ESR1. The hypothesis tested is that active, but not inactive, ESR1 fusions identified in metastatic patients induce ET resistance and metastasis by predictable reprogramming of gene expression (Aim 1), of protein kinase expression (Aim 2), and by recruitment of a common set of coactivators (Aim 3). We have made great strides in this first year period for Aims 1 and 2, and begun experiments for Aim 3. Briefly, in Aim 1, we have established functional rules that predict which ESR1 fusions are active drivers by identifying a 24-gene expression signature that active ESR1 fusions promote. In Aim 2, we have discovered that the RET receptor tyrosine kinase has elevated expression when active ESR1 fusions are expressed in breast cancer cells and that an inhibitor of RET reduces the growth of these cells, both <i>in vitro</i> and in patient-derived xenograft (PDX) tumors <i>in vivo</i> .					
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1. INTRODUCTION:

Our overarching **objectives** are to functionally characterize a previously unknown subclass of metastatic estrogen receptor- α positive (ER+), endocrine therapy (ET)-refractory breast cancers that contain in-frame *ESR1* fusions and define the rules to distinguish active from inactive fusions and to define new targeted therapies to reduce the tumor growth driven by active ESR1 fusions. Based on our published and preliminary data, we **hypothesize** that active *ESR1* fusions identified in advanced ER+ breast cancer patients induce ET resistance and metastasis by predictable reprogramming of protein kinase expression and by recruitment of a common set of coactivators, both of which can be therapeutically targeted. We will test this hypothesis in three Aims: Aim 1 is focused on defining a gene expression signature activated by active, but not inactive, ESR1 fusions; Aim 2 is focused on identifying kinase expression patterns altered by active ESR1 fusions and testing kinase inhibitors for breast cancer cell growth reduction; and Aim 3 is focused on defining transcriptional coactivators recruited to target genes by active ESR1 fusions and testing coactivator inhibitors for breast cancer cell growth reduction. This project was funded with Dr. Matthew Ellis as the PI, but Dr. Charles Foulds has become the PI and is responsible for the remaining molecular, cellular, and animal experiments. In year 1, we have made great strides and a manuscript describing our work on Aim 1 has been published by *Cancer Research* with another manuscript soon to be submitted on Aim 2 this year. We additionally have received a provisional patent based on the study published on Aim 1 experiments (see “Products”). Our task-specific progress is detailed below in the Accomplishments section.

2. KEYWORDS:

ESR1 gene fusion, endocrine therapy resistance, metastatic breast cancer, estrogen receptor- α (ER α), reprogramming, gene expression signature, protein kinase, transcriptional coactivator

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Per our approved SOW, this proposal has three specific aims (**major goals**): In **Aim 1**, we will establish functional rules that predict which ESR1 fusions are active drivers of drug resistance and metastasis. In **Aim 2**, we will investigate ESR1 fusion-activated downstream kinases for therapeutic targeting. In **Aim 3**, we will identify common coactivators recruited by ESR1 fusion proteins as therapeutic targets. We have made significant progress on Aim 1 and Aim 2 and have begun experiments on Aim 3 that will be discussed in more detail below.

*Important milestones listed in the approved SOW for **Aim 1** were:*

1) New in-frame ESR1 fusion stably expressing breast cancer cell lines created for subsequent in vitro analysis in Task 2. We proposed to create and functional study 10 new ESR1 fusions each year for a total of 30. We have already studied 15 (see *Cancer Research* paper, **Appendix**), so year 1 goal was surpassed, and we have more ESR1 fusions to study in subsequent years.

2) An “active ESR1 fusion gene signature” will be developed in year 1 and further refined and tested each year for prediction of new active ESR1 fusions from acquired clinical RNA-seq data. From

RNA-sequencing of active versus inactive ESR1 fusions (15 in total), we have defined a 24-gene expression signature that active ESR1 fusions induce (see *Cancer Research* paper, **Appendix**), so year 1 goal was met.

3) The *in vivo* effects of active *ESR1* fusions on breast tumor growth and metastasis will be defined. Here we proposed to transplant 5 active ESR1 fusions or wild-type ESR1 control T47D-luciferase cell lines into the fat pads of three-week old NOD/SCID ovariectomized female mice and measure primary tumor volumes and metastasis to distant organs. As we just defined which of 15 ESR1 fusions are active (10 out of the 15) (see *Cancer Research* paper, **Appendix**), we are now prepared to perform these experiments in year 2.

Important milestones listed in the approved SOW for Aim 2 were:

1) Identification of a “common” set of kinases activated in response to *ESR1* fusion expression. Here we proposed to perform kinase inhibitor pulldown (KIP) assays on 10 ESR1 fusions expressed in breast cancer cells per grant year. In year 1, we have profiled all 15 ESR1 fusions for which we did RNA-sequencing in Aim 1. From this analysis, we defined a subset of receptor tyrosine kinases (RTKs) that had elevated expression in active versus inactive ESR1 fusions. Thus, this goal was surpassed.

2) Efficacy of RET and IGFR1 kinase inhibition on *ESR1* fusion-driven tumorigenesis will be tested *in vivo*. Here we proposed to test these inhibitors on WHIM18 patient-derived xenograft (PDX) that naturally expresses the active ESR1-YAP1 fusion, as well as one active ESR1 fusion expressed in T47D cells tagged with luciferase. From cell line studies of multiple active ESR1 fusions, we found that the anti-IGFR1 antibody (Ganitumab) inhibited their growth at very high IC₅₀'s (>26 μ M). Thus, we focused on testing the RET inhibitor, pralsetinib, versus the CDK4/6 inhibitor palbociclib, which is the standard-of-care for advanced ER+ breast cancers (in which active ESR1 fusions are identified). We have acquired data for WHIM18 PDX and PDX organoids that pralsetinib is just as effective as palbociclib in reducing tumor cell growth, but the combination of both inhibitors did not provide any further benefit. Thus, we have met year 1 goal for WHIM18 PDX *in vivo* study. As we just defined which of 15 ESR1 fusions are active (10 out of the 15) (see *Cancer Research* paper, **Appendix**), we are now prepared to perform T47D cell-based xenograft drug tests in year 2.

Important milestones listed in the approved SOW for Aim 3 were:

1) Identification of “common” coactivators interacting with different ESR1 fusion proteins and their functionality. Here we proposed to perform ERE DNA pulldown- mass spectrometry assays of 10 ESR1 fusions per grant year. We have already made nuclear extracts from many of the ESR1 fusion expressing T47D cells and conducted pilot ERE DNA pulldowns with candidate-based immunoblotting and found SRC-2 and SRC-3 were recruited by active ESR1 fusions tested. With this data in hand, we are now prepared to execute this task in year 2.

2) Efficacy of coactivator inhibition on active *ESR1* fusion-driven tumorigenesis will be tested *in vivo*. As we just defined which of 15 ESR1 fusions are active (10 out of the 15) (see *Cancer Research* paper, **Appendix**) in year 1, it was important to first test coactivator inhibitor efficacy on T47D cell lines expressing active ESR1 fusions. In year 1, we did this for two steroid receptor coactivator (SRC) inhibitors (SI-2, SI-12) that reduce the activity of all three SRC/p160 activators. We found they strongly inhibited the viability of cells expressing active ESR1 fusions (IC₅₀'s from 1.2-4.7 nM), but not human primary mammary epithelial cells (IC₅₀'s of >395 nM). With this data in hand, we are now prepared to execute testing of SI-12 in WHIM18 and one T47D-based xenograft model in year 2.

What was accomplished under these goals?

Aim 1 accomplishments:

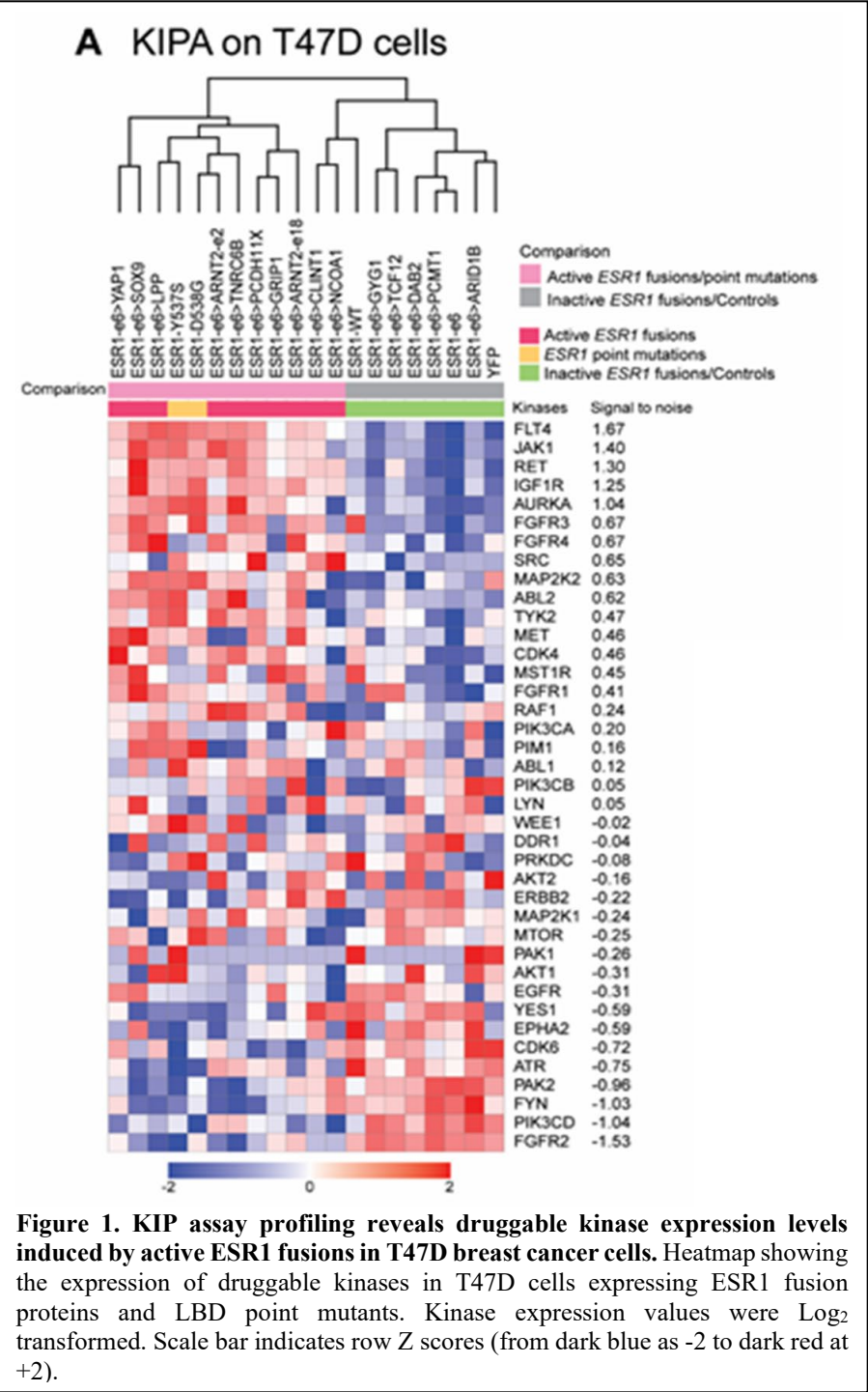
1) Major activities: Briefly, we functionally characterized 15 total in-frame ESR1 fusions in T47D and MCF7 ER+ breast cancer cell lines (please see *Cancer Research* paper in the **Appendix** as cited). This was done by first making lentiviral stable expression cell lines (please see Figure 2A, Supplementary Figure 1A, Supplementary Figure 5A and 5B). Next we tested “activity” of each ESR1 fusion by whether it prompted cell growth (as measured by an alamarBlue assay) in the absence of estradiol (E2) and presence of the selective ER α degrader, fulvestrant. We further tested whether these ESR1 fusions might enhance cell motility/migration in “wound healing” assays and invasion through Matrigel. We found 10 ESR1 fusions out of the 15 were active in these assays (please see Figures 1B, 1C, Supplementary Figures 1B-1D and 2, 5C-5E), which were ESR1-e6>YAP1, ESR1-e6>PCDH11X, ESR1-e6>SOX9, ESR1-e6>ARNT2-e18, ESR1-e6>ARNT2-e2, ESR1-e6>LPP, ESR1-e6>NCOA1, ESR1-e6>CLINT1, ESR1-e6>GRIP1, and ESR1-e6>TNRC6B. Four inactive fusions in both T47D and MCF7 cells were ESR1-e6>GYG1, ESR1-e6>PCMT1, ESR1-e6>ARID1B, and ESR1-e6>TCF12. Interestingly, ESR1-e6>DAB2 was inactive in T47D cells but active in MCF7 cells. Based on RNA-sequencing of four active ESR1 fusions versus three inactive fusions and negative controls (cells expressing YFP protein minus E2, cells expressing only the ESR1-e6, cells overexpressing wild-type (WT) ESR1 minus E2), we were able to show that active ESR1 fusion proteins upregulate expression of estrogen response and EMT genes (Figure 3) and through filtering for ER and EMT-related genes, we defined a 24-gene expression signature that was elevated in cells expressing active ESR1 fusions versus inactive fusions and control cells (Figure 4). We initially validated the signature for response in cells expressing additional ESR1 fusions (identified in another patient cohort) and found that our initial score cutoff accurately identified 5/6 active ESR1 fusions, as well as the active ESR1 ligand-binding domain point mutants (Y537S and D538G) (Figure 5). We next tested whether the gene signature would accurately predict the presence of an active ESR1 fusion and ESR1 ligand-binding mutants in ER+ PDX mouse models (that Dr. Ellis has developed over the years). We observed that our cell line based gene signature score cutoff was predictive of ESR1 mutant activity as higher scores were seen with WHIM18 (ESR1-e6>YAP1 active fusion), as well as WHIM PDX tumors that expressed active ESR1 point mutants (e.g., WHIM43, D538G; WHIM20, Y537S). Finally, we showed the 24-gene signature had prognostic value in ER+ metastatic breast cancer patients in the MET500 study. We observed higher gene signature score in RNA-seq of patients expressing an active ESR1 fusion (ESR1-e6>ARNT2-e18) or ESR1 point mutants (such as Y537S and D538G) (Figure 7). We thus termed this 24-gene expression signature “MOTERA” for “Mutant or Translocated ER Alpha”.

2) Specific objectives: Here we set forth to accomplish the three “Major Tasks” in the approved SOW, which are **1)** To identify new in-frame ESR1 fusions (10 new ones/grant year); **2)** To test whether ESR1 fusions are transcriptionally active to develop an “active ESR1 gene signature; and **3)** To test effects of active ESR1 fusion expression in promoting breast tumorigenesis *in vivo*.

3) Significant results: We set forth to test at least 10 ESR1 fusions for biological activity and found 10/15 were active in assays described above. This accomplished Major Task 1. Then we developed the 24-gene MOTERA signature that we validated as accurately predicting the presence of an active ESR1 fusion or point mutation in breast cancer cells, PDX models, and human metastatic breast cancer patient cohort. This accomplished Major Task 2. We have more work to do in year 2 to

accomplish Major Task 3, as we first had to define which ESR1 fusions that were found in metastatic ER+ breast cancer patients were indeed active drivers and not merely passenger mutations.

4) **Other achievements:** As noted below in the “**Products**” section, the discovery of the MOTERA gene signature has been filed as a USPTO provisional patent with the Baylor Licensing Group.



immunoblotting.

Aim 2 accomplishments:

1) **Major activities:** The goal of Aim 2 is to identify if a common set of protein kinases expression levels were induced when an active ESR1 fusion was expressed in an ER+ breast cancer cell. To this end, we employed kinase inhibitor pulldown (KIP) assays in which small molecule kinase inhibitors were first covalently bound to sepharose beads and then incubated with whole cell (or later PDX tumor lysates) to allow kinase binding followed by washes to remove unbound proteins. Label-free mass spectrometry then was used for protein identification.

We first did KIP assay in the above T47D cell lines expressing the different ESR1 fusions whose biological activity we firmly established (**Figure 1**). We discovered a subset of receptor tyrosine kinases (RTKs) had elevated expression levels in active versus inactive ESR1 fusions, such as FLT4, RET, IGF1R, and FGFR3/4, as well as JAK1 kinase. We validated the mass spectrometric data by

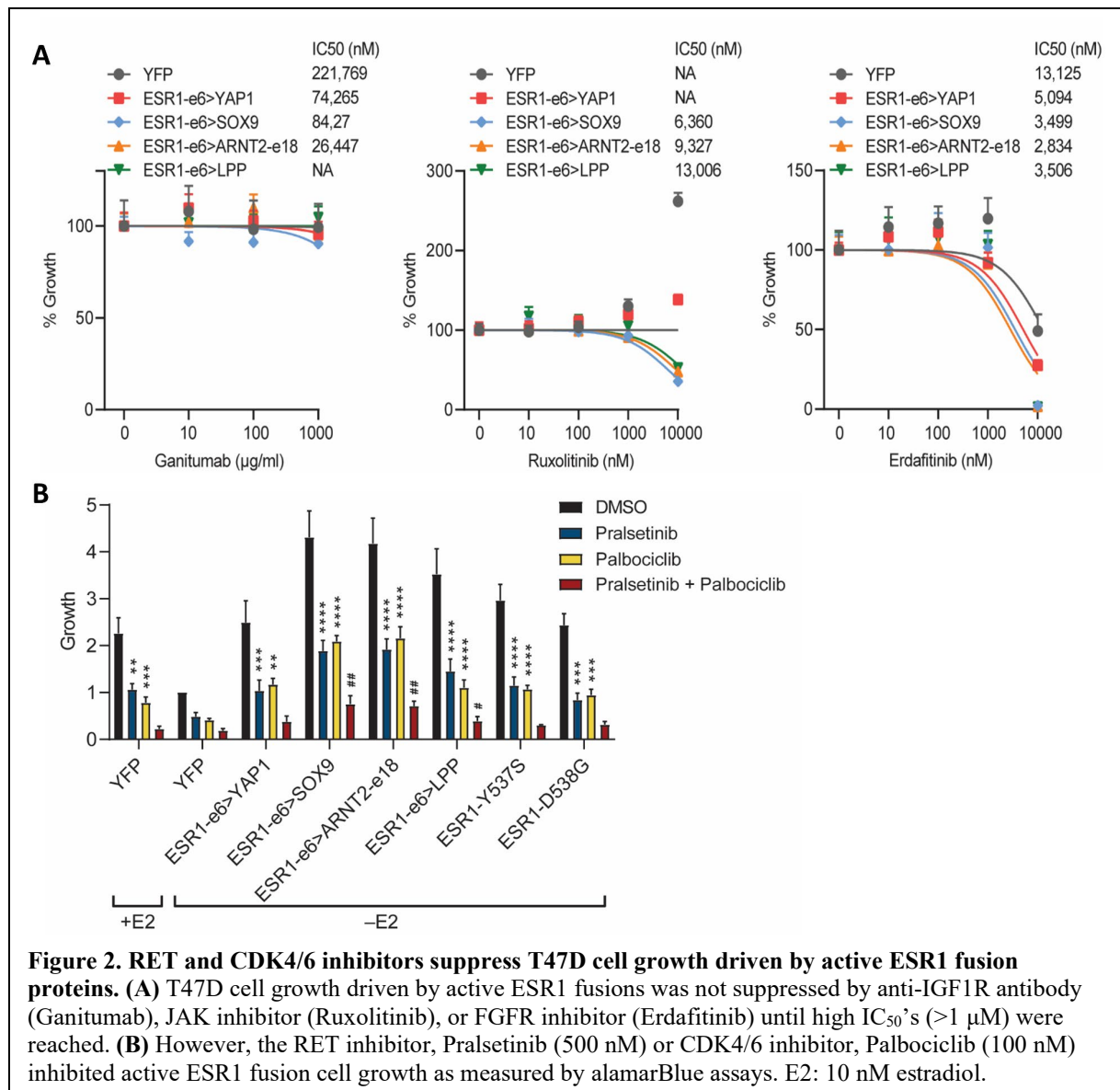
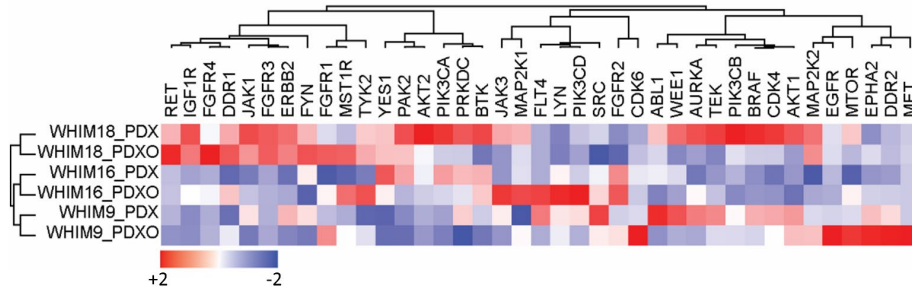


Figure 2. RET and CDK4/6 inhibitors suppress T47D cell growth driven by active ESR1 fusion proteins. (A) T47D cell growth driven by active ESR1 fusions was not suppressed by anti-IGF1R antibody (Ganitumab), JAK inhibitor (Ruxolitinib), or FGFR inhibitor (Erdafitinib) until high IC₅₀'s (>1 μM) were reached. (B) However, the RET inhibitor, Pralsetinib (500 nM) or CDK4/6 inhibitor, Palbociclib (100 nM) inhibited active ESR1 fusion cell growth as measured by alamarBlue assays. E2: 10 nM estradiol.

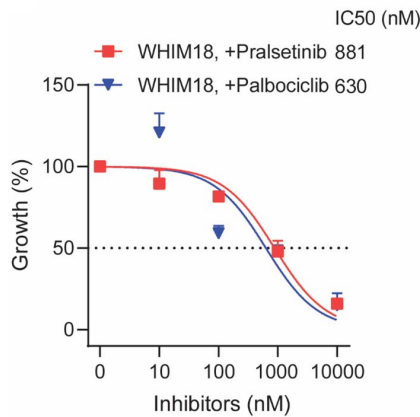
We next tested the effect of FDA-approved inhibitors to each of the above mentioned kinases for an effect on cell viability. IGF1R, pan-JAK, and pan-FGFR inhibitors displayed poor effects (much greater than 1 μM concentration was needed to inhibit growth (**Figure 2A**)). However, we found that the RET inhibitor, pralsetinib (FDA-approved for mutated *RET* lung and thyroid cancers and also called BLU-667, Subbiah, V *et al.* Cancer Discov. 2018;8(7):836-849. PMID: 29657135) at 500 nM significantly reduced the cell viability of both active ESR1 fusions and point mutants (Y537S, D538G), which was comparable to that achieved the CDK4/6 inhibitor palbociclib at 100 nM (**Figure 2B**).

We further validated the effects of pralsetinib and palbociclib on PDX 3D organoid *ex vivo* and PDX tumor growth *in vivo*. By using KIP assays, we first confirmed that the RTKs and JAK1 that we observed elevated in T47D cells expressing active ESR1 fusions were also elevated in WHIM18 (expressing the ESR1-e6>YAP1 fusion) as compared to WHIM9 or WHIM16 (both expressing WT

A KIPA on PDX and PDXO



B WHIM18 PDXO (CellTiter-Glo)



C WHIM9 PDXO (CellTiter-Glo)

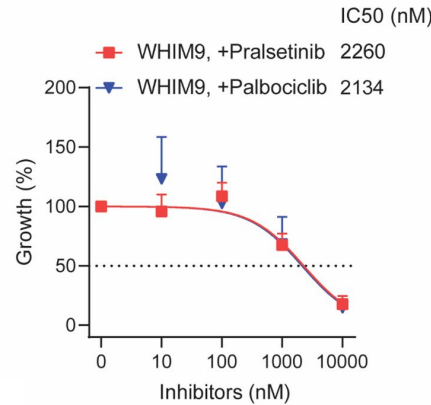
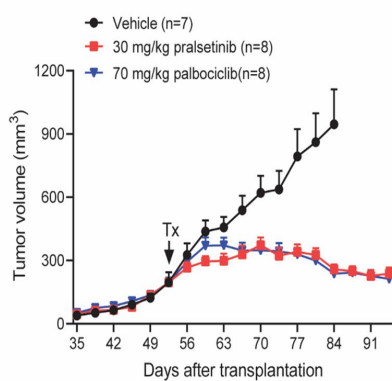


Figure 3. ER α + PDXO models recapitulate the kinome profile observed in matching PDX tumors (A) and RET and CDK4/6 inhibition suppresses ESR1 fusion-driven PDXO growth (B and C).

A WHIM18 growth



B WHIM18 body weight

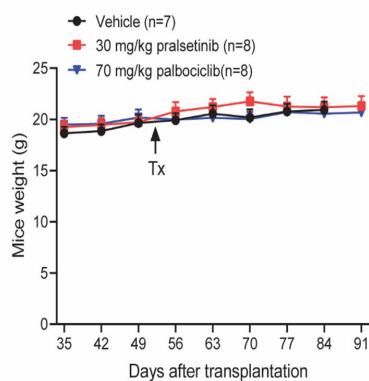


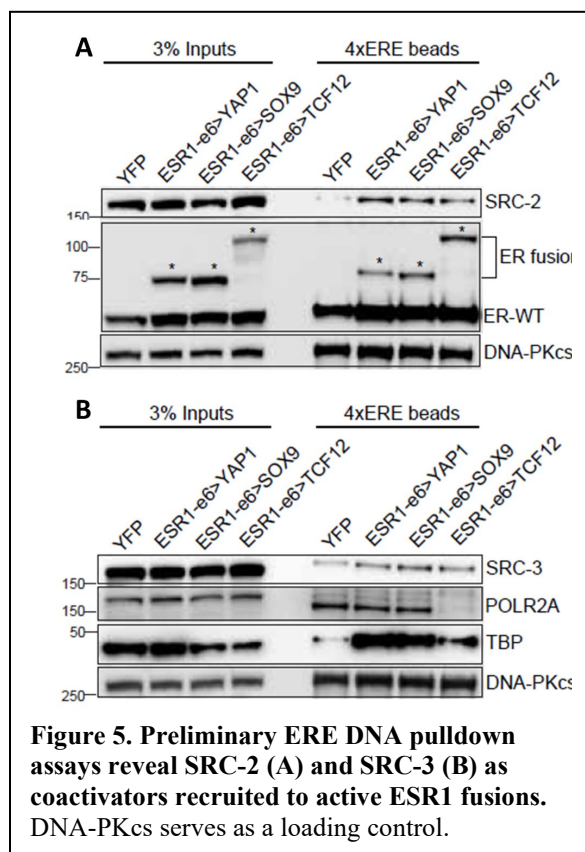
Figure 4. RET and CDK4/6 inhibitors suppress PDX tumor growth driven by an active ESR1 fusion. (A) Tumor volumes of WHIM18 PDX (mean $\pm$ SEM, n in each arm indicated) were measured daily. Arrow indicates the start of treatments (Tx), including vehicle, pralsetinib, or palbociclib containing chow. (B) Body weight of WHIM18 PDX mice. One-way ANOVA followed by Tukey's test was used to compare each treatment to vehicle arm (* p <0.05, ** p <0.01, *** p <0.001, and **** p <0.0001).

ESR1) PDX tumors and organoids derived from these PDX tumors (PDXO) (Figure 3A). We determined IC₅₀ values of the RET or CDK4/6 inhibitor in WHIM18 PDXO (Figure 3B) versus WHIM9 PDXO (Figure 3C) by use of an established organoid viability assay (CellTiter-Glo 3D, Promega). Consistent with WHIM18 PDXO expressing more RET than WHIM9 PDXO, we observed a 2.6x smaller IC₅₀ for pralsetinib in WHIM18 than WHIM9 organoids. Likewise, palbociclib was 3.4x more effective in WHIM18 than WHIM9 organoids. Consistent with our PDXO cell viability data, we observed both pralsetinib (30 mg/kg in chow) and palbociclib (70 mg/kg in chow) to be equally effective in reducing tumor growth in WHIM18 PDX mice (Figure 4A) without an effect on mouse body weight (as a proxy for overt toxicity) (Figure 4B).

2) Specific objectives: Here we set forth to accomplish the two “Major Tasks” in the approved SOW, which are **4) To identify kinases activated by expression of new identified ESR1 fusions (10 new ones/grant year) and 5) To pre-clinical test the efficacy of inhibition of above kinases on ESR1 fusion-driven tumorigenesis.**

3) Significant results: We set forth to perform KIP assay on at least 10 ESR1 fusions expressed in T47D cells to test if active ESR1 fusions reprogrammed kinase expression as compared to inactive. As seen in **Figure 1**, we accomplished Major Task 4 for year 1 by assaying 15 fusions and defining a subset of elevated kinases (some RTKs and JAK1). Then we tested FDA-approved inhibitors to the kinases with elevated expression to see if cell viability would be enhanced. As seen in **Figure 2**, only a RET inhibitor (pralsetinib) and a CDK4/6 inhibitor (palbociclib) displayed significant decreased T47D cell viability at drug concentrations less than 1 μ M. We then validated these inhibitors in PDX organoid models of an active ESR1 fusion model with high RET expression (WHIM18) versus a WT ESR1 model with lower RET expression (WHIM9) (**Figure 3**) and in WHIM18 PDX tumors in vivo (**Figure 4**). This accomplished Major Task 5 for year 1. We have more work to do in year 2 to perform KIP assay on additional new ESR1 fusions expressed in T47D cells and testing the effect of RET and CDK4/6 inhibition on ESR1 fusion-driven tumor growth and metastasis in T47D-luciferase cell line xenografts (one active ESR1 fusion/grant year). This second *in vivo* model was delayed in year 1, as we first had to define which kinases were elevated in our existing 10 active ESR1 fusions.

4) Other achievements: We are writing up the data shown in this report (plus additional data not in the report) to finish a manuscript for submission to an AACR-based journal. Below are more details on this paper to be submitted within the next month:



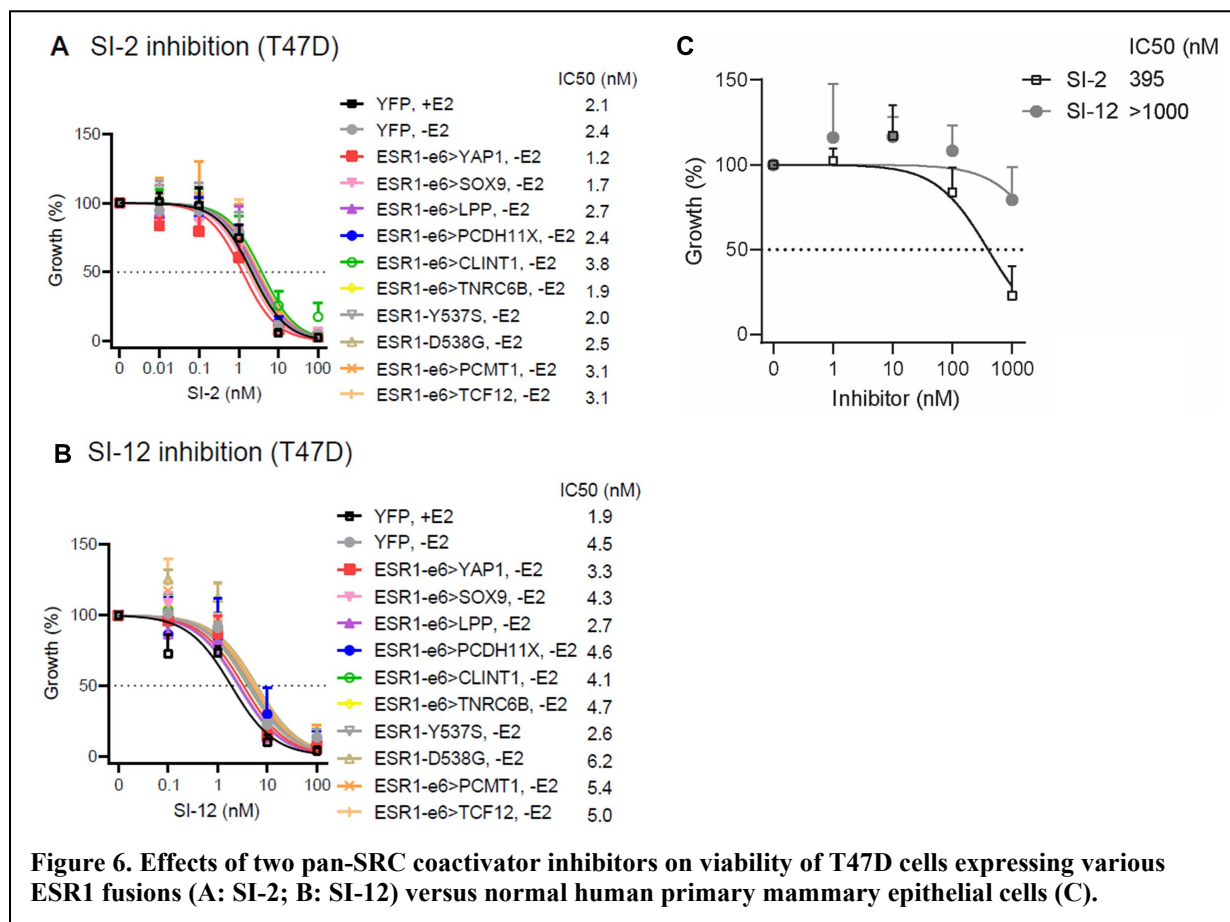
Xuxu Gou, Beom-Jun Kim, Meenakshi Anurag, Jonathan T. Lei, Matthew V. Holt, Meggie N. Young, Diana Fandino, Craig T. Vollert, Purba Singh, Mohammad A. Alzubi, Lacey E. Dobrolecki, Michael T. Lewis, Alana L. Welm, Shunqiang Li, **Charles E. Foulds, Matthew J. Ellis.** Targeting kinome reprogramming in ESR1 fusion-driven metastatic breast cancer.

Aim 3 accomplishments:

1) Major activities: The goal of Aim 3 is to identify common transcriptional coactivators recruited by active ESR1 fusion proteins as therapeutic targets. To accomplish this, we will employ ERE DNA pulldown assays (developed by Dr. Foulds) and used previously to profile transcriptional coactivators recruited to WT ESR1 bound to E2 (Foulds CE, *et al.* Mol Cell. 2013;51(2):185-99. PMID: 23850489) or ESR1 point mutants (Y537S, D538) or the ESR1-e6-YAP1 fusion (Gates LA, *et al.*, Oncogene. 2018 37(33):4581-4598. PMID: 29748621). Here we will make nuclear extracts of active ESR1 fusions and inactive ones and then incubate them with four copies of biotinylated estrogen response elements (4xEREs)

fused to a minimal RNA polymerase II (RNA pol II) promoter immobilized on streptavidin magnetic beads. Non-specifically bound proteins are washed away and the remaining bound proteins are identified by label-free mass spectrometry (MS) for protein identification. In year 1, we have majorly focused on Aims 1 and 2 with clear progress made, but Aim 3 will be more of a focus in year 2. We have made nuclear extracts from many different ESR1 fusion expressing cell lines and have proof-of-principle data by immunoblotting that at least two SRC/p160 coactivators are recruited to active ESR1 fusions (ESR1-e6>YAP1 and ESR1-e6>SOX9) (Figure 5). Interestingly, ESR1-e6>TCF12 fusion protein that has a transcription factor as a C-terminal partner was able to recruit the two SRCs. However, consistent with its lack of transcriptional activity (see *Cancer Research* paper, **Appendix**), it blocks RNA pol II large subunit (POLR2A) recruitment and reduces TATA-binding protein (TBP) to the minimal promoter (**Figure 5B**). In year 2, we will further validate this unexpected mechanistic result by performing chromatin immunoprecipitation (ChIP) assays in ESR1-e6>TCF12 cells versus active ESR1 fusion expressing T47D cells. Consistent with the approved SOW, we will perform MS on ERE DNA pulldowns from 10 distinct ESR1 fusion expressing cell lines in year 2.

Because we observed two SRC/p160 coactivators recruited to two active ESR1 fusion proteins in **Figure 5**, we next tested the effect of pan-SRC coactivator inhibitors (SI-2/SI-12) on viability of T47D cells expressing active ESR1 fusions. We observed in all cell lines that both SRC coactivator inhibitors strongly inhibited growth (IC_{50} 's < 7 nM) (**Figures 6A and 6B**). To rule out that these SRC inhibitors were grossly cytotoxic, we determined their IC_{50} 's in human primary mammary epithelial cells (HMECs) and observed much less toxicity (IC_{50} 's: 395 nM and >1 μ M) (**Figure 6C**). These data



are important to inform subsequent animal xenograft experiments, as they support the use of SI-12 and not SI-2.

2) Specific objectives: Here we set forth to accomplish the two “Major Tasks” in the approved SOW, which are **6)** To identify a set of “common” coactivators recruited by different in-frame ESR1 fusion proteins to ERE-containing DNA and **7)** To test effects of active ESR1 fusion protein-interacting coactivator inhibition on ESR1 fusion-driven tumorigenesis.

3) Significant results: We have provided proof-of-principle that the ERE DNA pulldown assays will detect transcriptional coactivators (and other RNA pol II machinery), which is critical for Major Task 6 to be done in year 2 with MS instead of only immunoblotting. We have also shown that two pan-SRC coactivator inhibitors reduce the viability of all ESR1 fusion expressing T47D cells with low nanomolar IC₅₀'s. Importantly, two SRC coactivator inhibitors are much less lethal to normal HMECs. These data are foundational for Major Task 7, as we will need to test these inhibitors in both WHIM18 PDXO and PDX *in vivo* models in year 2.

4) Other achievements: Nothing to report.

What opportunities for training and professional development has the project provided?

Xuxu Gou was a Ph.D. student in the Ellis laboratory during year 1 grant support. As such, she was afforded “training” activities by one-on-one work with her mentors. Namely, Dr. Anurag aided Ms. Gou in development of the 24-gene expression signature in Aim 1; Dr. Kim taught her how to perform KIP assays and analyze the data for Aim 2; and Dr. Foulds taught her how to perform ERE DNA pulldown assays for Aim 3. Drs. Ellis and Foulds worked with her to improve her manuscript and deal with requested reviewer revisions. This was very important for her future scientific career. Ms. Gou was afforded “professional development” activities by grant funding support for her abstract submissions to conferences as listed in the below section “**Products**”.

How were the results disseminated to communities of interest?

The results of our work on Aim 1 have been published in *Cancer Research* as an “open access” paper. We have additionally shared our data on Aims 1 and 2 at conferences listed in the below section “**Products**”.

What do you plan to do during the next reporting period to accomplish the goals?

As cited above in the “milestones” discussion, here is the list of experiments “to do” in year 2 to accomplish the three major goals:

1) For Aim 1, we will functionally characterize more ESR1 fusions in year 2. Dr. Foulds has acquired a new MTA with Tempus to share genomic coordinates of newly identified *ESR1-e6* gene fusions. Two of these, ESR1-AKAP12 and ESR1-PLEKHG1, have also been reported by Adrian Lee's laboratory (Hartmaier RJ et al. Ann Oncol. 2018;29(4):872-880. PMID: 29360925) and thus appear recurrent. Thus, we will prioritize their functional study by constructing the fusions in a lentiviral vector to infect T47D cells. We will also define the “MOTERA” gene signature score in these new ESR1 fusion expressing cells. We will transplant 5 active ESR1 fusions or wild-type ESR1 control T47D-luciferase cell lines into the fat pads of three-week old NOD/SCID ovariectomized female mice and measure primary tumor volumes and metastasis to distant organs.

2) For Aim 2, we will test to see if the same kinases have elevated expression in the above mentioned new ESR1 fusion cell lines by performing more KIP assays. To complement our WHIM18 PDX drug studies *in vivo*, we will perform T47D cell-based xenograft drug tests in year 2.

3) For Aim 3, we perform ERE DNA pulldown- mass spectrometry assays of at least 10 ESR1 fusions in year 2. We have already tested the effects of SRC/p160 coactivator inhibitors on the viability of T47D cells expressing active ESR1 fusions, so in year 2 we will execute testing of the most potent (yet least cytotoxic) SI-12 in WHIM18 and one T47D-based xenograft model.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

During year 1, we defined a 24-gene expression signature (composed of ER α target and epithelial-mesenchymal transition (EMT) genes) induced by active ESR1 fusions, but not inactive fusions that represented a major accomplishment for Aim 1. This work was published in *Cancer Research* and supported a provisional patent through Baylor College of Medicine for diagnosis of future patients that may express an active ESR1 fusion (and ESR1 ligand-binding domain point mutant as well) as a major innovation. We are currently testing the utility of the signature genes in formalin-fixed paraffin-embedded (FFPE) patient-derived xenograft (PDX) breast tumor samples to correctly identify the presence (WHIM18, ESR1-YAP1) or absence (WHIM9, wild-type ESR1) of an ESR1 fusion. If successfully, we will then extend the signature to analysis of metastatic ER+ breast cancer patient biopsies.

We also identified a subset of receptor tyrosine kinases (RTKs) that displayed elevated expression in active ESR1 fusion expressing breast cancer cells and found that one of them, RET, conferred a therapeutic vulnerability in these cancers. We found that the RET inhibitor (Pralsetinib) was able to significantly reduce the tumor growth mediated by active ESR1 fusions. This work was done for Aim 2 and will be submitted this year. The impact of this work is that there are no targeted therapies yet for active ESR1 fusion driven breast cancers, and this RET inhibitor may represent one from our pre-clinical experiments.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Dr. Ellis left Baylor College of Medicine to be Senior Vice-President of Early Oncology at AstraZeneca on 03-07-2022. We notified the grants official (Jamie A. Shortall) and requested Dr. Charles Foulds be the PI on the remaining two years left on this grant. This request was kindly granted by the grants official effective on 03-15-2022 (E01 Modification P0001). Dr. Foulds will

provide oversight to this grant and prepare all progress reports including year 1. He has worked with Dr. Ellis on the original grant proposal and daily monitoring of the research from its original funding date.

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

During year 1 of this grant, we published the following peer-reviewed paper:

Gou X, Anurag M, Lei JT, Kim BJ, Singh P, Seker S, Fandino D, Han A, Rehman S, Hu J, Korchina V, Doddapaneni H, Dobrolecki LE, Mitsiades N, Lewis MT, Welm AL, Li S, Lee AV, Robinson DR, **Foulds CE, Ellis MJ**. Transcriptional Reprogramming Differentiates Active from Inactive ESR1 Fusions in Endocrine Therapy-Refractory Metastatic Breast Cancer. Cancer Res. 2021;81(24):6259-6272. PMID: 34711608. Acknowledgement of federal support: Yes.

Books or other non-periodical, one-time publications.

During year 1, Xuxu Gou wrote her Ph.D. dissertation at Baylor College of Medicine entitled: "Proteogenomic reprogramming of ESR1 fusion-driven metastatic breast cancer and targeted therapy". 2022.

We also published the following abstract from the listed scientific conference:

Xuxu Gou, Meenakshi Anurag, Jonathan T. Lei, Sinem Seker, Adrian V. Lee, Dan R. Robinson, **Matthew J. Ellis**. The integration of a structure-function rule and a transcriptional signature to assign ESR1 fusion activity in metastatic breast cancer [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2021; 2021 Apr 10-15 and May 17-21. Philadelphia (PA): AACR; Cancer Res 2021;81(13_Suppl):Abstract nr 742. <https://doi.org/10.1158/1538-7445.AM2021-742>.

Other publications, conference papers and presentations.

Abstracts not published but presented at various listed conferences include:

1) Gou, X; Anurag, M; Lei, JT; Kim BJ; Singh, P; Seker, S; Fandino, D; Al-Zubi, M; Han, A; Rehman, S; Hu, J; Korchina, V; Doddapaneni, H; Dobrolecki, LE; Mitsiades, N; Lewis, MT; Welm, AL; Li, S; Lee, AV; Robinson, DR; **Foulds, CE; Ellis, MJ**. Proteogenomic Reprogramming of ESR1 Fusion Metastatic Breast Cancer and Targeted Therapy. 17th Annual Dan L Duncan Comprehensive Cancer Center Symposium at Baylor College of Medicine, Houston, TX, 2021.

2) Xuxu Gou, Meenakshi Anurag, Jonathan T. Lei, Beom-Jun Kim, Purba Singh, Sinem Seker, Diana Fandino, Mohammad Al-Zubi, Airi Han, Saif Rehman, Jianhong Hu, Viktoriya Korchina, Harsha Doddapaneni, Lacey E. Dobrolecki, Nicholas Mitsiades, Michael T. Lewis, Alana L. Welm, Shunqiang Li, Adrian V. Lee, Dan R. Robinson, **Charles E. Foulds, Matthew J. Ellis**. Proteogenomic Reprogramming of ESR1 Translocated Metastatic Breast Cancer and Targeted Therapy. 16th Annual Breast Cancer Education Conference at Baylor College of Medicine, Houston, TX, 2021.

3) Xuxu Gou, Beom-Jun Kim, Meenakshi Anurag, Matthew V. Holt, Meggie N. Young, Jonathan T. Lei, Diana Fandino, Craig T. Vollert, Purba Singh, Mohammad A. Alzubi, Lacey E. Dobrolecki, Michael T. Lewis, Alana L. Welm, Shunqiang Li, **Charles E. Foulds, Matthew J. Ellis**. Targeting kinome reprogramming in ESR1 fusion-driven metastatic breast cancer. ASCO Annual Meeting, Chicago, IL, 2022.

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

As noted above, we filled a provisional patent on the 24-gene expression signature termed “MOTERA” that were defined from work on Aim 1 that was published in *Cancer Research* with Baylor Licensing Group (BLG) as #21-066, 04/15/2021 disclosed entitled “Mutant or Translocated Estrogen Receptor Alpha (MOTERA) Signature”, USPTO, Application #63254890.

- **Other Products**

From work on Aim 1 published in *Cancer Research*, we have the following “other products”:

1) RNA-sequencing data of T47D breast cancer cells expressing wild-type *ESR1*, two *ESR1* ligand-binding domain mutations (Y537S, D538G), and 15 different *ESR1* gene fusions that is publically-available at GEO with accession number GSE191158.

2) T47D and MCF7 breast cancer cell lines expressing 13 distinct *ESR1*-exon 6 (*e6*) gene fusions to the following partner genes: *NCOA1*, *LPP*, *CLINT1*, *GRIPI*, *TNRC6B*, *SOX9*, *ARNT2* (exon 2), *ARNT2* (exon 18), *PCMT1*, *DAB2*, *GYGI*, *ARID1B*, and *TCF12*.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name	Project role	ORCID ID	Person Mon worked	Project contribution	Funding support
Ellis, Matthew	PI (from 03/15/21-03/07/22)	0000-0002-8467-8534	0.60	Responsible for overseeing the overall design, execution, and progress of the proposed research plan. Wrote the <i>Cancer Research</i> paper.	This grant.
Foulds, Charles	Co-Investigator (from 03/15/21-03/07/22); PI currently	0000-0003-4908-1473	4.8	Dr. Foulds collaborated with Dr. Ellis' graduate student Xuxu Gou in creation of <i>ESR1</i> fusion cell lines described in Aim 1. He will oversee PDX and T47D xenograft experiments using coactivator inhibitors proposed in Aim 3. He will work with Xuxu Gou on performing ERE DNA pulldowns in Aim 3. Wrote the <i>Cancer Research</i>	This grant.

				paper.	
Anurag, Meenakshi	Co-Investigator	0000-0003-4379-5192	1.8	Responsible for identification of <i>ESR1</i> fusions from TEMPUS patient RNA-seq datasets in Aim 1.	This grant
Kim, Beom-Jun	Co-Investigator	0000-0003-3109-8170	1.2	Will conduct the KIP and phosphoproteomic experiments in Aim 2.	This grant
Fandino, Diana	Research Tech	N/A	8.4	Responsible for care of the mice in proposed xenograft experiments in all three Aims.	This grant.
Gou, Xuxu	Research Asst. GS	0000-0003-2318-778X	4.0	Responsible in Aim 1 for creating and characterizing all <i>ESR1</i> fusion breast cancer cell lines. Helped Dr. Kim with KIP and phosphoproteomic assays in Aim 2. Will help Dr. Foulds do ERE DNA	This grant

				pull-down assays in Aim 3.	
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Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

As noted above, Dr. Ellis was PI during the first year of the grant. He will not be on the grant in subsequent years. Dr. Foulds assumes the PI role for years 2 and 3.

Updated Other Support Attached.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

9. APPENDICES:

1) Please see the paper and supplementary data on Aim 1 work that was recently published by *Cancer Research*:

Gou X, Anurag M, Lei JT, Kim BJ, Singh P, Seker S, Fandino D, Han A, Rehman S, Hu J, Korchina V, Doddapaneni H, Dobrolecki LE, Mitsiades N, Lewis MT, Welm AL, Li S, Lee AV, Robinson DR, **Foulds CE, Ellis MJ**. Transcriptional Reprogramming Differentiates Active from Inactive ESR1 Fusions in Endocrine Therapy-Refractory Metastatic Breast Cancer. *Cancer Res.* 2021;81(24):6259-6272. PMID: 34711608.



Transcriptional Reprogramming Differentiates Active from Inactive ESR1 Fusions in Endocrine Therapy-Refractory Metastatic Breast Cancer

Xuxu Gou^{1,2}, Meenakshi Anurag^{1,3}, Jonathan T. Lei^{1,4}, Beom-Jun Kim^{1,3}, Purba Singh¹, Sinem Seker¹, Diana Fandino¹, Airi Han^{1,5}, Saif Rehman⁶, Jianhong Hu⁷, Viktoriya Korchina⁷, Harshavardhan Doddapaneni⁷, Lacey E. Dobrolecki¹, Nicholas Mitsiades^{3,8,9}, Michael T. Lewis^{1,8,9,10}, Alana L. Welm¹¹, Shunqiang Li¹², Adrian V. Lee^{13,14}, Dan R. Robinson^{15,16}, Charles E. Foulds^{1,3,9}, and Matthew J. Ellis^{1,2,3,8,9}

ABSTRACT

Genomic analysis has recently identified multiple *ESR1* gene translocations in estrogen receptor alpha-positive (ER α ⁺) metastatic breast cancer (MBC) that encode chimeric proteins whereby the ESR1 ligand binding domain (LBD) is replaced by C-terminal sequences from many different gene partners. Here we functionally screened 15 ESR1 fusions and identified 10 that promoted estradiol-independent cell growth, motility, invasion, epithelial-to-mesenchymal transition, and resistance to fulvestrant. RNA sequencing identified a gene expression pattern specific to functionally active ESR1 gene fusions that was subsequently reduced to a diagnostic 24-gene signature. This signature was further examined in 20 ER α ⁺ patient-derived xenografts and in

55 ER α ⁺ MBC samples. The 24-gene signature successfully identified cases harboring *ESR1* gene fusions and also accurately diagnosed the presence of activating *ESR1* LBD point mutations. Therefore, the 24-gene signature represents an efficient approach to screening samples for the presence of diverse somatic *ESR1* mutations and translocations that drive endocrine treatment failure in MBC.

Significance: This study identifies a gene signature diagnostic for functional ESR1 fusions that drive poor outcome in advanced breast cancer, which could also help guide precision medicine approaches in patients harboring *ESR1* mutations.

Introduction

The majority of breast cancers (~70%) are initially diagnosed as estrogen receptor-alpha positive (ER α ⁺) and are dependent on 17 β estradiol (E2) for growth (1). Thus, endocrine therapies (ET) either induce estrogen deprivation, for example, through aromatase inhibition (AI), or directly target the ER α ligand binding domain (LBD) with selective ER modulation (e.g., tamoxifen) or degradation (e.g., fulvestrant; ref. 1). However, acquired ET resistance is common, and is often associated with somatic mutations in the gene encoding ER α , *ESR1*. The most extensively studied examples are point mutations in the LBD that result in ER α proteins with ligand-independent activity. Common examples include Y537S and D538G (2, 3). These mutations typically arise after patients have undergone extensive endocrine treatment and can be present in up to 40% of patients with ER α ⁺ metastatic breast cancer (MBC; refs. 4, 5).

Emerging evidence indicates that chromosomal translocations involving the *ESR1* gene can also drive ET resistance through the formation of chimeric transcription factors (TF) with constitutive activity (6, 7). The first described example was an ESR1-e6>YAP1 fusion detected by whole-genome sequencing and RNA sequencing (RNA-seq) in samples from a patient with rapid onset ET resistance (6). The fusion protein was encoded by an interchromosomal translocation event that brought *ESR1* exons 1 to 6 (ESR1-e6) on chromosome (chr) 6q into the *YAP1* locus on chr11q, thereby replacing the entire LBD with transactivation domain (TAD) sequences from this Hippo pathway transcriptional coactivator (CoA). A patient-derived xenograft (PDX) established from the patient's tumor (WHIM18) also exhibited ET resistance. We subsequently identified another in-frame exon 6 fusion, ESR1-e6>PCDH11X in a male patient with ER α ⁺

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Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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MBC as a result of a chr6q>Xq translocation. This second example was a harbinger of complexity to come, because *PCDH11X* encodes a protocadherin without known transcriptional functions. Both fusions not only induced ET-resistant tumor growth but also increased lung metastasis in xenograft mice models (7). Another in-frame ESR1-e6 fusion, ESR1-e6>NOP2, was detected in a primary tumor but was found to be transcriptionally inactive (7). This example suggested that the mere presence of an ESR1-e6 fusion that generates a stable chimeric protein is insufficient evidence that an ET-resistance driver has been identified.

Multiple additional ESR1-e6 fusions have now been identified from ER⁺ MBC patients. In a study by Lee and colleagues, three ESR1-e6 fusions (ESR1-e6>DAB2, ESR1-e6>GYG1, and ESR1-e6>SOX9) were shown to activate an estrogen response element (ERE)-driven luciferase reporter construct in transfected HEK293 cells (8). In the MET500 study (9), three in-frame fusions (ESR1-e6>ARNT2-e18, ESR1-e6>PCMT1, and ESR1-e6>ARID1B) were identified in samples from MBC patients and were provided to us for functional studies in a pre-publication personal communication. To further investigate the significance of in-frame ESR1-e6 fusion genes, each example was screened for ET-resistance induction *in vitro*, defined as E2-independent and fulvestrant-resistant growth and increased motility, in two ER⁺ breast cancer cell lines (T47D and MCF7). RNA-seq was undertaken to understand the transcriptional reprogramming induced by active ESR1 fusions. We subsequently trained a 24-gene signature that was characteristic of the presence of an active ESR1 fusion as compared with an inactive fusion or wild-type (WT) ESR1. In the effort to validate this signature in ER⁺ PDXs and in clinical samples, activating LBD point mutations were discovered to also induce this gene expression signature. These data suggest that despite the remarkable diversity of mutations in the *ESR1* gene, these somatic events converge on a common pathogenic transcriptional reprogramming mechanism to drive poor outcome and ET resistance in MBC.

Materials and Methods

Cell culture

Growth conditions for T47D (ATCC; cat #HTB-133, RRID: CVCL_0553) and MCF7 (ATCC; cat #HTB-22, RRID: CVCL_0031) cells are described in Lei and colleagues (7) and detailed in Supplementary Information. ER α ligands (E2 and fulvestrant) were purchased from Sigma (E4389) and Selleckchem (S1191), respectively.

Subcloning of ESR1 mutants into a lentiviral expression vector

Lentiviral vectors expressing C-terminal HA-tagged yellow fluorescent protein (YFP), truncated ESR1-e6, ESR1-WT, ESR1-Y537S, ESR1-D538G, ESR1-e6>YAP1, and ESR1-e6>PCDH11X were previously described (6, 7). HA-tagged cDNAs encoding the new ESR1-e6 fusions in this study were constructed in a similar fashion as detailed in Supplementary Information.

Generation of lentiviral stable ESR1-mutant expressing cell lines

Lentivirus were produced as described (6) by cotransfecting the above *ESR1* cDNA lentiviral vectors with the packaging plasmids pMD2.G (RRID: Addgene_12259) and psPAX2 (RRID: Addgene_12260) into HEK293T (ATCC; cat #CRL-3216, RRID: CVCL_0063) cells using Lipofectamine 2000 (Invitrogen, cat #11668-027). Transduced breast cancer cells were selected with 2 μ g/mL puromycin (Sigma; cat #P8833) for 7 days. Expression of various ESR1 proteins was validated using immunoblotting.

Immunoblotting and immunoprecipitation

Cells were harvested and whole-cell lysates were prepared in RIPA lysis buffer as described (7) or in MIB lysis buffer (10) supplemented with 1 $\times$ protease inhibitors and 1 $\times$ phosphatase inhibitors (Roche) by sonication for 2 minutes. To make ER⁺ PDX tumor lysates, frozen PDX tumors were cryopulverized with a Covaris CP02 Pulverizer, and then protein was extracted in MIB lysis buffer with sonication. Protein concentration determination and SDS-PAGE (20 μ g protein per lane) were performed as described (7). Immunoblotting of nitrocellulose membranes was performed as described (7). Primary and HRP-conjugated secondary antibodies used are listed in the Supplementary Information.

Immunoprecipitation was performed as described (7), using 2 mg of lysates from hormone-deprived T47D cells with or without E2 treatment (100 nmol/L for 45 minutes). Lysates were incubated with 2 μ g anti-HA tag antibody (Santa Cruz Biotechnology; cat #sc-7392, RRID: AB_627809) or mouse IgG (Cell Signaling Technology; cat #61656, RRID: AB_2799613) control, followed by capture of antibody-antigen complexes with protein A magnetic beads (Bio-Rad, cat #1614013) as described (7). Immunoprecipitated proteins, as well as 20 μ g of whole-cell lysates (1% inputs), were analyzed by immunoblotting.

Cell growth, motility, and invasion assays

Cell growth assays of different ESR1 fusion protein expressing breast cancer cells that were first hormone-deprived and then subsequently treated either with 100 nmol/L fulvestrant in the presence or absence of 10 nmol/L E2 for 7 to 10 days were performed in 96-well plates using an alamarBlue assay as described (7). Cell growth reading values were normalized to that of control YFP cells, -E2.

Cell motility was detected using a scratch wound assay of hormone-deprived stable cells in a 96-well ImageLock plate (Essen BioScience) that were pretreated for 2 hours with mitomycin C (50 ng/mL for T47D and 200 ng/mL for MCF7; Sigma, M4287) before wounding as described (7). Wound images were acquired every 6 hours for 72 hours by an IncuCyte camera (Essen Bioscience) in a cell culture incubator. Relative wound densities (RWD) were calculated as density in the wound area relative to that outside the wound area to account for confounding proliferation.

The cell invasion assay was performed and analyzed in a similar manner to the scratch wound assay except that cells were plated on Matrigel-coated plate. After the scratch was generated on cell monolayer, 50 μ L Matrigel solution was added to the wells, thus filling the scratch region and 100 μ L of additional culture media containing mitomycin C.

RNA-seq and analysis

Different *ESR1* cDNA stably expressing T47D cell lines were cultured in CSS media for 5 days followed by treatment with or without 10 nmol/L E2 for 2 days. RNA was isolated using the RNeasy Mini Kit (QIAGEN; cat #74106) and treated with DNase (QIAGEN; cat #79254) to remove genomic DNA. The Genomic and RNA Profiling (GARP) Core at BCM confirmed concentration (using a NanoDrop spectrophotometer) and integrity (using an Agilent Bioanalyzer). The GARP core then made mRNA libraries and performed sequencing on an Illumina NovaSeq 6000 sequencing instrument as described in detail in Supplementary Information. For RNA-seq on isolated ER⁺ PDX tumors, frozen PDX tumors were cryopulverized as above and total RNA was isolated using the RNeasy kit. RNA-seq was performed at the Human Genome Sequencing Center at BCM as described in detail in Supplementary Information.

For RNA-seq analysis, paired-end 150 bp reads were aligned to the hg19 (GRCh37) reference genome using RSEM v1.2.31 (RSEM, RRID: SCR_013027; ref. 11) and Bowtie 2 (12). Transcripts per million values calculated by RSEM were $\log_2$ transformed and subjected to heatmap generation using Morpheus (<https://software.broadinstitute.org/morpheus>; Morpheus, RRID:SCR_014975). Unsupervised hierarchical clustering and identification of differentially expressed genes in active ESR1 fusion protein expressing cells to cells expressing inactive fusions and controls are described in Supplementary Information.

Whole-exome sequencing and analysis

DNA was isolated from the ER⁺ PDX tumors using a QIAamp DNA Mini Kit (QIAGEN; cat #51304). WES data were generated by the Human Genome Sequencing Center at BCM using the Illumina platform as described in detail in Supplementary Information. Tools used for somatic ESR1 LBD gene variant calling were Strelka2, Mutect2, and CARNAC (v 0.2.5b9) as described in Supplementary Information. ESR1-e6>YAP1 fusion was detected in WHIM18 previously (6).

Reverse transcription-quantitative PCR

RNA was isolated from hormone-deprived stable T47D cells as above with concentration determined using a NanoDrop spectrophotometer. One-step RT-qPCR was conducted using 50 ng RNA incubated with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad; cat #1725274), iScript reverse transcriptase (Bio-Rad; cat #170-8891) and 0.5 μ m primers (Sigma) as described (7). All samples were run in triplicate on a CFX96 thermal cycler (Bio-Rad).

Immunofluorescence

Immunofluorescence was performed of different HA-tagged ESR1 fusion proteins expressed in hormone-deprived T47D cells as described (7). These proteins were detected using an anti-HA antibody (Cell Signaling Technology; cat #2367, RRID:AB_10691311, 1:50) and goat anti-mouse IgG secondary antibody (Alexa Fluor 568, Molecular Probes; cat #A-11004, RRID:AB_2534072, 1:1,000) as described in Supplementary Information. Nuclei were detected by DAPI staining as described (7).

PDX models

The PDX models were previously described (6, 13, 14). All animal procedures were approved by the Institutional Animal Care and Use Committee at BCM (protocol #AN-6934). Two- to 3-mm pieces from PDX tumors were engrafted into cleared mammary fat pads of 3- to 4-week-old ovariectomized SCID/beige mice (Charles River). Mice were randomized to receive sterile drinking water with or without 8 μ g/mL E2 supplementation ($n = 7$ –16 per PDX line per arm). Tumor volumes were measured by caliper every 3–4 days, and were calculated by $V = 4/3 \times \pi \times (\text{width}/2)^2 \times (\text{length}/2)$. Mice were sacrificed when tumors reached 1.5 cm³ or at the study endpoint. Tumors were harvested and frozen in liquid nitrogen for storage. Additional information on BCM and HCI PDX models is available at pdxportal.research.bcm.edu/.

Gene signature and ROC curve analysis

The signature performance was calculated as follows: Accuracy = $(TP + TN)/(TP + TN + FP + FN)$, sensitivity = $TP/(TP + FN)$, specificity = $TN/(TN + FP)$, in which TP, true positive; TN, true negative; FP, false positive; FN, false negative. Receiver operating

characteristic (ROC) curve analysis was performed using “pROC” package in R (15).

ERE DNA pulldown assays

These assays were modified from the established protocol of HeLa cell nuclear extract (NE) supplemented with recombinant estrogen receptors (16, 17). Briefly, nuclear extracts were made from T47D cell lines expressing YFP or different ESR1 fusion proteins (15–25 15-cm dishes used) exactly as published (18). Pulldown assays used 1 mg of T47D cell NE to resuspend 60 μ L Dynabeads M-280 Streptavidin that was prebound to 3 μ g biotinylated 4xERE-E4 921 bp DNA. Incubation occurred at 4°C with gentle rotation for roughly 2 hours, followed by pelleting beads with a magnetic rack and quick washes as described (16, 17). Final beads were resuspended in 30 μ L 2xSDS-sample buffer, boiled, and 30% of the final supernatants were loaded onto 4% to 15% gradient SDS-PAGE gels. After transfer to nitrocellulose, immunoblots were probed with N-terminal ER α (Millipore; cat #04-820, RRID:AB_1587018) or DNA-PKcs (Santa Cruz Biotechnology; cat #sc-5282, RRID:AB_2172848) antibodies with ECL-based signal detection on a Bio-Rad Imaging System.

Statistical tests and analyses of publicly available data

Unpaired two-tailed Student *t* test and ANOVA were performed with GraphPad Prism 9 (GraphPad Prism, RRID:SCR_002798), as indicated in the figure legends. *P* values less than 0.05 were considered statistically significant. The protein domains with functional information in **Figs. 1B** and **5A** were extracted from UniProt Knowledgebase (19). RNA-seq data derived from ER⁺ MBC along with ESR1 mutation status ($n = 55$) were downloaded from the MET500 web portal (<https://met500.path.med.umich.edu/>). Cases with an ESR1 mRNA expression >1 FPKM were considered as ER⁺, using a previously described criterion (20).

Data availability

RNA-seq data from T47D cells and WES and RNA-seq data from ER⁺ PDX tumors in this study are being submitted to Gene Expression Omnibus (GEO). Until an accession number is provided, these raw data are available upon request from the corresponding author.

A full description of all methods and reagents can be found in Supplementary Information.

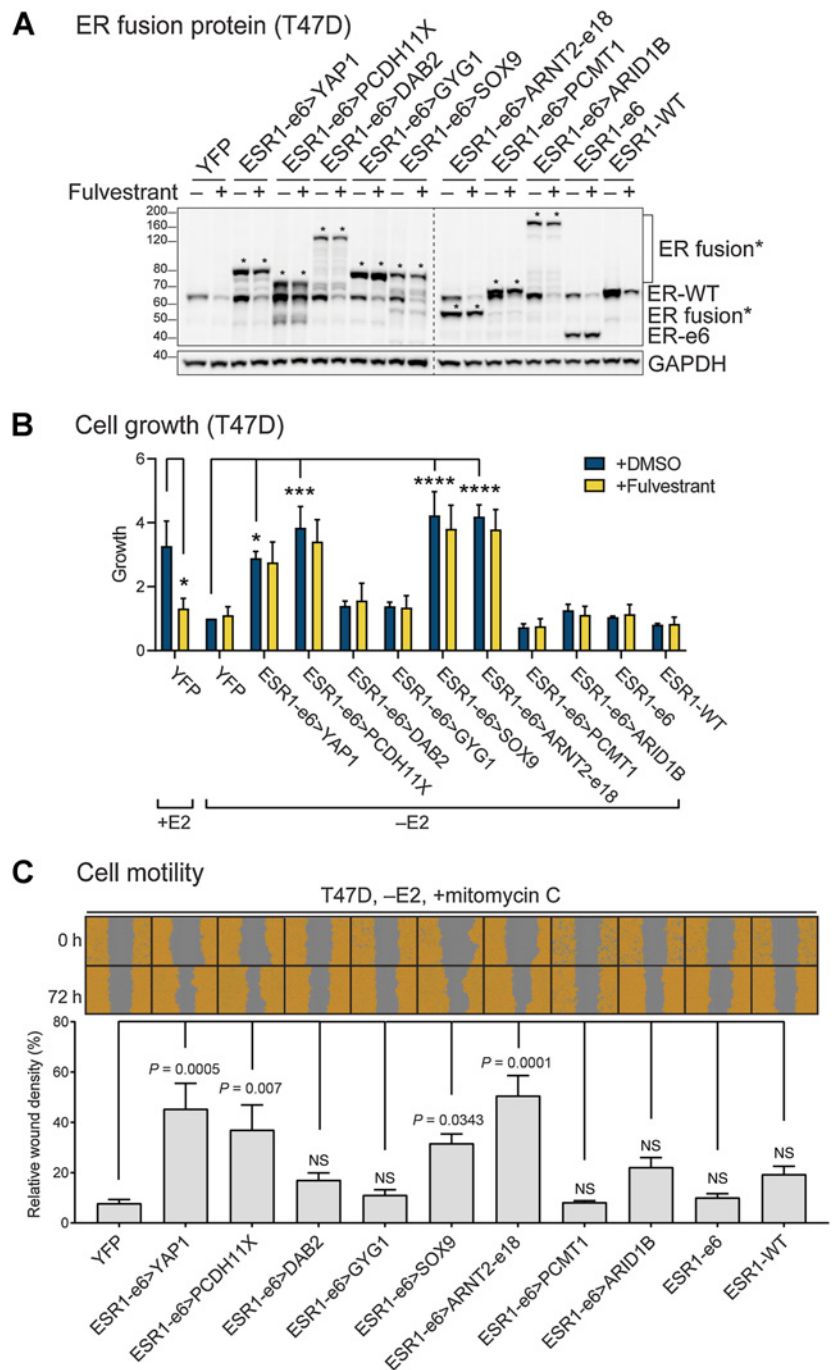
Results

A subset of in-frame ESR1-e6 fusions identified in ER⁺ MBC patients drives ET-resistant growth and promotes hormone-independent motility and invasion of ER⁺ breast cancer cells

We initially studied six newly identified in-frame ESR1 fusions detected in samples from MBC patients and compared them to the ESR1-e6>YAP1 and ESR1-e6>PCDH11X examples we described previously (7). Some fusion examples arose from interchromosomal translocations, such as ESR1-e6>DAB2, ESR1-e6>GYG1, ESR1-e6>SOX9 (Lee Laboratory; ref. 8), and ESR1-e6>ARNT2-e18 (Robinson, D. personal communication). Two other fusions were formed by rearrangements within chromosome 6, ESR1-e6>PCMT1 and ESR1-e6>ARID1B (D. Robinson, personal communication; **Fig. 1A**). All six examples followed a structure established by the original ESR1-e6>YAP1 fusion whereby the first six exons of ESR1 were fused in-frame to C-terminal partner genes, completely replacing the ER α LBD with an alternative C-terminus. We noted two classes functionally, (i) TF and transcription coactivator (CoA) fusions or (ii) fusions with

Figure 2.

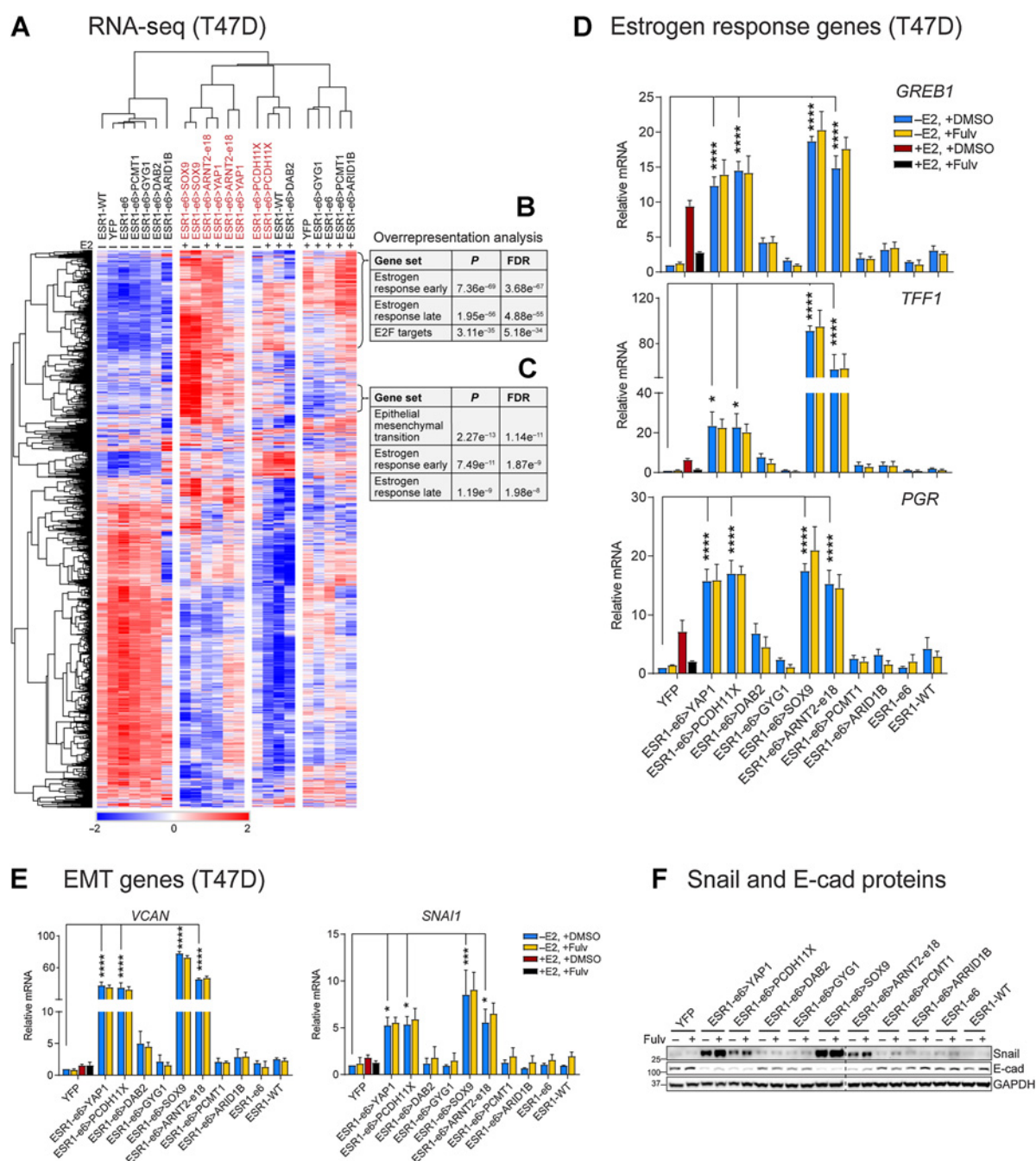
ESR1 fusion proteins drive ET-resistant growth and promote hormone-independent motility of ER⁺ breast cancer cells. **A**, Immunoblotting of ER α and ESR1 fusion proteins with an N-terminal ER α antibody in lysates made from hormone-deprived stable T47D cells. Asterisks indicate ER fusion proteins. GAPDH serves as a loading control. The dashed line indicates two separate blots that were conducted at the same time. The representative image is from three independent experiments. **B**, Cell growth was assayed in hormone-deprived stable cells (mean $\pm$ SEM; $n = 3$). One-way ANOVA followed by Dunnett multiple comparisons test was used to compare data of hormone-deprived ESR1 fusion expressing cells with YFP control cells in the vehicle (+DMSO) group. Two-way ANOVA followed by Bonferroni test was used for multiple comparisons for each stable cell line after 100 nmol/L fulvestrant treatment in the presence or absence of 10 nmol/L estradiol (E2). *, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$. See Supplementary Fig. S1B for the complete data. **C**, Cell motility was detected using scratch wound assays in hormone-deprived stable T47D cells, treated with mitomycin C to block proliferation (mean $\pm$ SEM; $n = 3$). Cells are pseudo-colored orange to aid visualization. One-way ANOVA followed by Dunnett multiple comparisons test was used to compare each stable T47D cell line with YFP control cells. NS, not significant.



Active ESR1 fusion proteins upregulate expression of estrogen response genes and EMT genes

To define the transcriptional profile driven by active ESR1 fusion proteins, RNA-seq was performed on T47D cells expressing these ESR1 fusion cDNAs as well as control (YFP, ESR1-e6, and ESR1-WT) cells in the presence and absence of E2. Hierarchical clustering showed that T47D cells expressing ESR1-e6>YAP1, ESR1-e6>PCDH11X, ESR1-e6>SOX9, and ESR1-e6>ARNT2-e18 fusions clustered distinctly from other ESR1-e6 fusions and control cells under E2-deprived conditions (-E2; Fig. 3A). All four active fusions demonstrated an expression pattern similar to control cells treated with E2, consistent

with potent hormone-independent transcriptional activation of "estrogen response" genes (Fig. 3B). Interestingly, active ESR1 fusion proteins also upregulated a cluster of genes that were not observed in the control cells stimulated by E2 (Fig. 3C). Overrepresentation analysis revealed a significant enrichment of "estrogen response" pathways and an epithelial-to-mesenchymal transition (EMT) signature specific to active ESR1 fusion proteins and thus consistent with data presented above on invasion and motility (Fig. 3B and C). The expression of three canonical estrogen response genes in stably transfected T47D cells was validated using RT-qPCR. ESR1-e6>YAP1, ESR1-e6>PCDH11X, ESR1-e6>SOX9, and ESR1-e6>ARNT2-e18

**Figure 3.**

Active ESR1 fusion proteins upregulate expression of estrogen response and EMT genes. **A**, Heatmap showing the differentially expressed genes in the T47D RNA-seq data. **B** and **C**, Active ESR1 fusions upregulate expression of two clusters of estrogen response (early and late) and EMT-related genes as indicated. **D** and **E**, Expression of estrogen response genes (*GREB1*, *TFF1*, and *PGR*) and EMT-related genes (*VCAN* and *SNAI1*) was measured by RT-qPCR in E2-depleted T47D cells treated with vehicle (+DMSO) or 100 nmol/L fulvestrant (+Fulv) in the absence (-E2) or presence (+E2) of 10 nmol/L E2 for 48 hours. Values were normalized to *GAPDH* mRNA, and relative expression was calculated as fold change to YFP, -E2 (mean $\pm$ SEM; $n = 3$). One-way ANOVA followed by Dunnett multiple comparisons test was used to compare each E2-depleted T47D cell line with YFP control cells (*, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$). Two-way ANOVA followed by Bonferroni test was used for multiple comparisons for each stable cell after 100 nmol/L fulvestrant treatment. **F**, Snail and E-cadherin proteins were measured by immunoblotting in E2-depleted cells treated with or without 100 nmol/L fulvestrant (Fulv). GAPDH protein served as a loading control. The dashed line indicates two separate blots that were conducted at the same time. The representative image is from three independent experiments.

significantly induced the expression of *GREB1*, *TFF1*, and *PGR* mRNA, all three known as direct ER α targets (22) (Fig. 3D), in a hormone-independent, fulvestrant-resistant manner compared with YFP controls. These transcriptionally active ESR1 fusion proteins also upregulated two EMT-related genes, *SNAIL1* (Snail), encoding a master TF that induces EMT (23) by transcriptional repression of epithelial genes such as *E-cadherin* (24), and *VCAN* (versican; Fig. 3E). The elevated expression of Snail protein and a corresponding decrease of E-cadherin (E-cad) were confirmed by immunoblotting (Fig. 3F). As expected, the expression of these genes were unaffected by fulvestrant treatment. The other ESR1-e6 fusion examples, ESR1-e6>DAB2, ESR1-e6>GYG1, ESR1-e6>PCMT1, and ESR1-e6>ARID1B, did not induce E2-independent activation of ER α target genes and EMT-related genes in T47D cells. The induction of Snail protein was also reproduced in MCF7 cells; however, ESR1-e6>PCDH11X displayed a minor upregulation compared with other transcriptionally active fusions (Supplementary Fig. S1A). Consistent with the observed MCF7 cell line-selective increase in cell growth and migration, ESR1-e6>DAB2 also upregulated Snail expression compared with YFP control and the inactive fusions. Consistent with above T47D cell line data, E-cadherin protein was reduced in MCF7 cells expressing active ESR1 fusion proteins (Supplementary Fig. S1A).

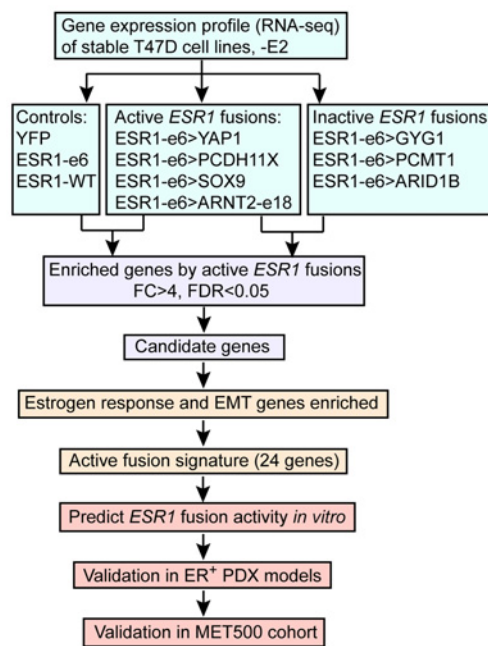
Additional experiments were conducted to demonstrate that inactive ESR1 fusion proteins enter the nucleus as the nuclear translocation

signal is preserved. Also, there is no biochemical evidence for heterodimer formation with WT ER α (Supplementary Fig. S3). These data imply that the inactive ESR1 fusion genes are not dominant negative, a result consistent with the normal E2-induced growth in inactive fusion-expressing cells.

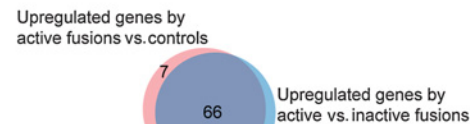
Active ESR1 fusion proteins induce a characteristic, hormone-independent transcriptional signature

The 3' partners of ESR1 fusion genes are highly diverse; consequently, their presence is only revealed by unbiased genomic techniques such as whole-genome sequencing or RNA-seq. These techniques are not routinely used clinically, and it is currently unknown how sensitive unbiased techniques are as screens for an ESR1 gene fusion event, because an orthogonal assay is required to determine sensitivity. Adding to diagnostic complexity, some ESR1 fusion proteins are inactive and therefore not clinically actionable. An *in vitro* assay such as the ones described above are feasible but difficult to conduct within a clinically useful time-frame. We therefore sought to develop a gene expression signature that is diagnostic for the presence of a transcriptionally active ESR1 fusion protein. RNA-seq was applied to T47D cells expressing ESR1 fusion cDNAs to identify genes that were selectively upregulated by the four transcriptionally active ESR1 fusion proteins as compared with: (i) three inactive ESR1 fusions and (ii) three controls (Fig. 4A). These two comparisons yielded an overlapping group of 66

A Workflow



B Enriched hallmark gene sets



Gene sets	Number of candidate genes in the hallmark	P	FDR
Estrogen response early	19	1.08 e ⁻²⁸	5.41 e ⁻²⁷
Estrogen response late	13	1.3 e ⁻¹⁷	3.26 e ⁻¹⁶
EMT	5	2.06 e ⁻⁵	3.43 e ⁻⁴

C Score comparison (training set)

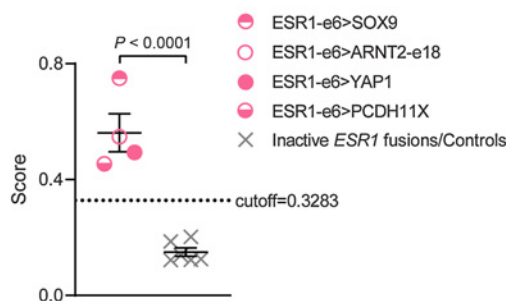


Figure 4.

Active ESR1 fusions program a unique, 24-gene transcriptional signature. **A**, Workflow to identify the gene signature to predict active fusions. FC, fold change; FDR, false discovery rate. **B**, Venn diagram showing overlapping upregulated genes by active ESR1 fusions compared with inactive fusions or control cells. The table below shows the top three Hallmark gene sets enriched in the candidate genes. **C**, Scatter plot showing signature scores of active ESR1 fusions (ESR1-e6>YAP1, ESR1-e6>PCDH11X, ESR1-e6>SOX9, and ESR1-e6>ARNT2-e18) compared with inactive fusions (ESR1-e6>GYG1, ESR1-e6>PCMT1, and ESR1-e6>ARID1B) and control cells (YFP, ESR1-e6, and ESR1-WT) all minus E2. A two-tailed *t* test was used to calculate statistical significance.

candidate genes with a fold change greater than 4 and a false discovery rate (FDR) less than 0.05. Overrepresentation analysis using Hallmark pathways from MSigDB (25, 26) identified candidate genes that were overrepresented in the estrogen response (early and late) and EMT gene sets (Fig. 4B). An active ESR1 fusion signature was then devised based on estrogen response and EMT genes, as these were the top two pathways modulated by expression of an active ESR1 fusion protein. Specifically, we identified 24 Hallmark genes, including 19 genes in the estrogen response set (*CHST8*, *MAPT*, *OLFM1*, *PDZK1*, *RASGRP1*, *MPPED2*, *GREB1*, *MYB*, *GFRA1*, *PGR*, *ELOVL2*, *ADCY1*, *NPY1R*, *TFF1*, *ACOX2*, *SGK1*, *STC2*, *CALCR*, and *KRT13*), two genes in the EMT gene set (*VCAN* and *COL3A1*), and three genes in both gene sets (*CXCL12*, *GJA1*, and *TGM2*). The expression of each gene was ranked by percentile within each sample and scores were computed as the mean percentile of the signature gene sets. ESR1 fusions were predicted as encoding active or inactive proteins according to the cutoff obtained by the ROC curve analysis (cutoff, 0.3283; Supplementary Fig. S4). In this training set, transcriptionally active ESR1 fusion proteins showed significantly higher scores as compared with inactive fusions and controls, as expected (Fig. 4C).

The 24-gene transcriptional signature predicts the *in vitro* activity of additional ESR1-e6 fusion genes

To validate the 24-gene ESR1 fusion activity signature, we studied seven additional ESR1 gene fusions published by Priestly and colleagues (27). These in-frame ESR1-e6 fusions were identified in ER⁺ MBC patients by whole-genome sequencing, including four fusions with TF or CoA partners, ESR1-e6>ARNT2-e2, ESR1-e6>LPP, ESR1-e6>NCOA1, and ESR1-e6>TCF12 (Fig. 5A). Another three fusions, analogous to *PCDH11X*, involved genes encoding protein-protein interaction motifs that serve nontranscriptional cellular functions, including ESR1-e6>CLINT1, ESR1-e6>GRIP1, and ESR1-e6>TNRC6B. The same approach as in Fig. 2 was taken to assess the function of these new fusions *in vitro*. All of the seven ESR1 fusion cDNAs expressed stable chimeric proteins in T47D and MCF7 cells (Supplementary Fig. S5A and S5B). Three fusions that involve TF/CoA partners, ESR1-e6>ARNT2-e2, ESR1-e6>LPP, and ESR1-e6>NCOA1, drove E2-independent and fulvestrant-resistant growth, as well as increased motility of T47D cells, when compared with the YFP controls (–E2, +DMSO; Supplementary Fig. S5C–S5E). Surprisingly, the fourth fusion, ESR1-e6>TCF12, which involves a TF in the basic helix–loop–helix (bHLH) E-box family, expressed a stable chimeric protein, but was inactive in both T47D and MCF7 cells (Supplementary Fig. S5C–S5E). The ESR1-e6>TCF12 fusion was able to bind to concatenated EREs in a pulldown assay similar to active fusion examples (ESR1-e6>YAP1, ESR1-e6>SOX9, and ESR1-e6>CLINT1; Supplementary Fig. S6), thus suggesting that the transcriptional inactivity of ESR1-e6>TCF12 was not due to lack of an ability to bind DNA.

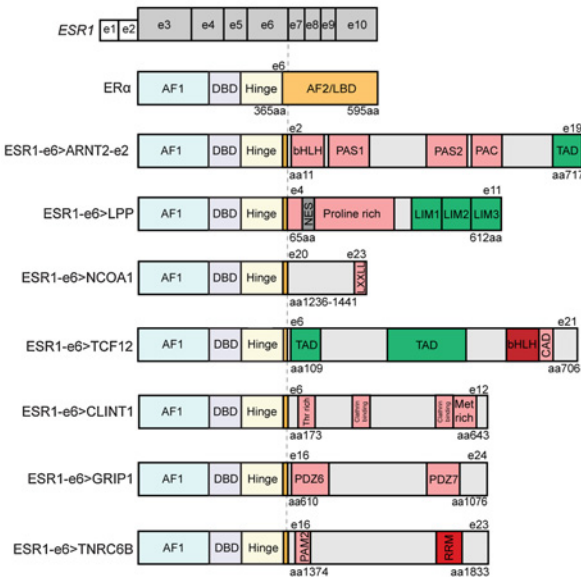
Three gene fusions that did not involve a known TF/CoA partner, ESR1-e6>CLINT1, ESR1-e6>GRIP1, and ESR1-e6>TNRC6B, but all demonstrated ET-resistant cell growth and enhanced E2-independent motility, although the effect of ESR1-e6>GRIP1 on proliferation was statistically marginal (Supplementary Fig. S5C–S5E). RNA-seq was then performed on RNA extracted from T47D cells that expressed each new ESR1 fusion protein, as well as on RNA from YFP control cells. In this experiment, we also included two common ESR1 LBD point mutations (Y537S and D538G) to compare the active ESR1 fusion signature with the transcriptional profile associated with known activating ESR1 point mutants. Five out of six active ESR1 fusions (ESR1-e6>ARNT2-e2, ESR1-e6>LPP, ESR1-e6>NCOA1, ESR1-

e6>CLINT1, and ESR1-e6>TNRC6B) demonstrated similar elevated expression of the 24-gene signature in sum, although there was some variability at the level of individual genes (Fig. 5B). ESR1-e6>GRIP1 induced lower expression of the 24-gene signature than other active fusion examples, consistent with its weaker activity in proliferation assays compared with the five other fusions studied. Interestingly, the two ESR1 LBD point mutant proteins expressed in T47D cells induced similar levels of gene expression from the 24-gene signature as active ESR1 fusion proteins, suggesting that despite different mutational mechanisms for ESR1 protein activation, LBD point mutants and translocated ERs activate a similar pathogenic transcriptional pattern (Fig. 5B). The mean signature scores of active ESR1 fusions and LBD point mutants were significantly increased compared with those of the inactive ESR1-e6>TCF12 fusion and YFP (endogenous ER α) control (Fig. 5C). As expected, the mean score of the weakly active ESR1-e6>GRIP1 fusion fell below the cutoff value. The validation statistics of the independent Priestley and colleagues (27) set showed an accuracy of 90.0% (specificity, 100%; sensitivity, 87.5%; Fig. 5C). Because the 24-gene signature was similarly induced by ESR1 LBD point mutants and active ESR1 fusion proteins, it was given the moniker “MOTERA” for Mutant or Translocated Estrogen Receptor Alpha.

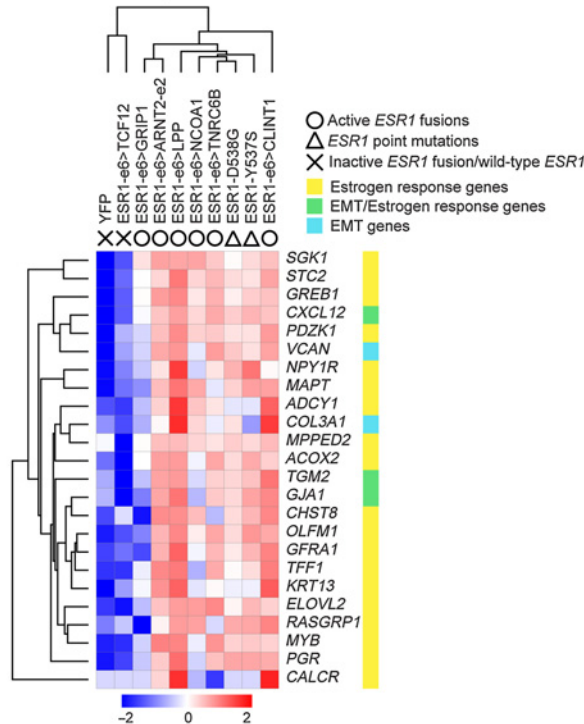
The MOTERA signature accurately predicts the presence and functional status of ESR1 mutations and gene fusions in ER⁺ PDX tumors and clinical samples.

To test the properties of the MOTERA signature in human tumors that naturally express either ESR1 gene fusions or mutations, we examined performance for ESR1 fusion or point mutation detection in a panel of 20 ER⁺ PDX tumors. The E2 dependence of each PDX tumor was evaluated in ovariectomized SCID/beige mice with or without 8 μ g/mL E2 in the drinking water (Fig. 6). ESR1 mutation status was determined by WES and gene expression was determined by RNA-seq under both plus estradiol (+E2) and minus estradiol (–E2) conditions. When tumors were completely E2 dependent, the –E2 transcriptome was established by replacing the +E2 water with control (–E2) water for one week and then harvesting the tumors. As expected, the MOTERA signature was highly expressed in the E2-independent WHIM18 PDX naturally expressing the ESR1–YAP1 fusion protein (6) (Fig. 7A), thus demonstrating a high degree of similarity between the experimental context of the ESR1-e6>YAP1 cDNA in T47D cells and the natural context in a PDX where this fusion was first identified. Consistent with T47D-based gene expression findings displayed in Fig. 5B, ET-resistant PDXs bearing a variety of ESR1 LBD point mutations also induced the MOTERA signature, confirming an overlap between the transcriptional properties of active ESR1 fusion proteins and LBD point mutants noted in T47D cell experiments (Fig. 7A). For example, the MOTERA signature score was enriched over the cutoff derived from the T47D training set in the cases of BCM15100, WHIM20, WHIM40, and HCI013 (all expressing ESR1–Y537S), WHIM37 and WHIM43 (expressing ESR1–D538G), WHIM24 (expressing ESR1–E380Q), WHIM27 (expressing ESR1–Y537N), and HCI005 and HCI007 (expressing ESR1–L536P) mutants (Fig. 7B). PDX tumors expressing ESR1-WT (HCI003, HCI011, BCM15057, BCM4888, BCM15034, BCM3277, BCM7441, WHIM9, and WHIM16) had MOTERA scores below the cutoff in low estradiol (–E2) conditions in each case (Fig. 7B). We note that the mean signature scores for ESR1-WT tumors increased with E2, consistent with some genes in the signature being E2-induced (Fig. 7A and B). Thus, as a screening tool, the MOTERA signature is likely to be more specific if the biopsy sample is taken while the patient is taking an AI or an antiestrogen. Paradoxically, the HCI013 PDX example harbors the

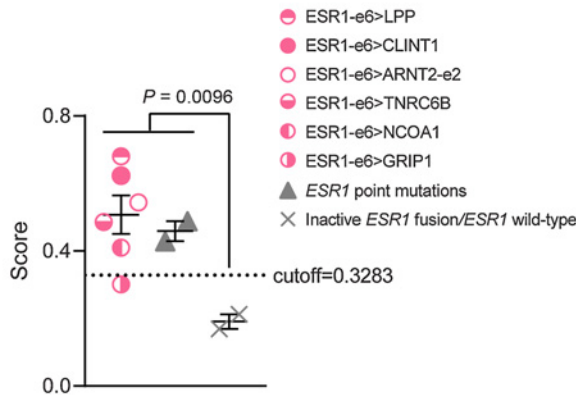
A ESR1-e6 fusions in Priestley et al.



B Gene signature expression



C Score comparison and signature performance (validation set)



Priestley et al. validation set		Activity confirmed by experiments	
		Active	Inactive
Activity predicted by the signature	Active	7	0
	Inactive	1	2

Accuracy: 90.0%
Sensitivity: 87.5%
Specificity: 100%

Figure 5. The MOTERA signature predicts activity of additional *ESR1* fusions identified in ER⁺ MBC patients. **A**, Seven additional *ESR1*-e6 fusions identified in Priestley et al. (27) are illustrated. These in-frame fusions possess a common structure as shown in **Fig. 1B**. Pink boxes represent protein-protein interactions, including the Per-Arnt-Sim (PAS) domain, PAC motif, LXXLL motif, class A specific domain (CAD), threonine-rich domain (Thr rich), methionine-rich domain (Met rich), PDZ domain, and PABPC1-interacting motif-2 (PAM2). Green boxes either represent transcriptional activation domains (TAD) or LIM zinc-binding (LIM) domains that provide coactivator function for LPP. The gray box represents a nuclear export signal (NES) in LPP. Red boxes represent the bHLH DNA binding domain and the RNA recognition motif (RRM). **B**, Heatmap showing the expression of the 24-gene signature in T47D cells expressing additional *ESR1* fusions and LBD point mutations (Y537S and D538G). Scale bar indicates row Z scores. **C**, Left, scatter plot showing signature scores of *ESR1* mutations (including fusions and LBD point mutations) and YFP control cells expressing endogenous ERα. Two-tailed *t* test was used to compare scores. The *ESR1*-GRIP1 fusion was the only active fusion that did not reach score significance. Right, confusion matrix to measure the performance of the signature to predict the activities of *ESR1* fusions. Accuracy is the proportion of correctly predicted events in all cases. Sensitivity is the ability of the signature to predict an active fusion event to be active. Specificity is the ability of the signature to predict an inactive fusion event to be inactive.

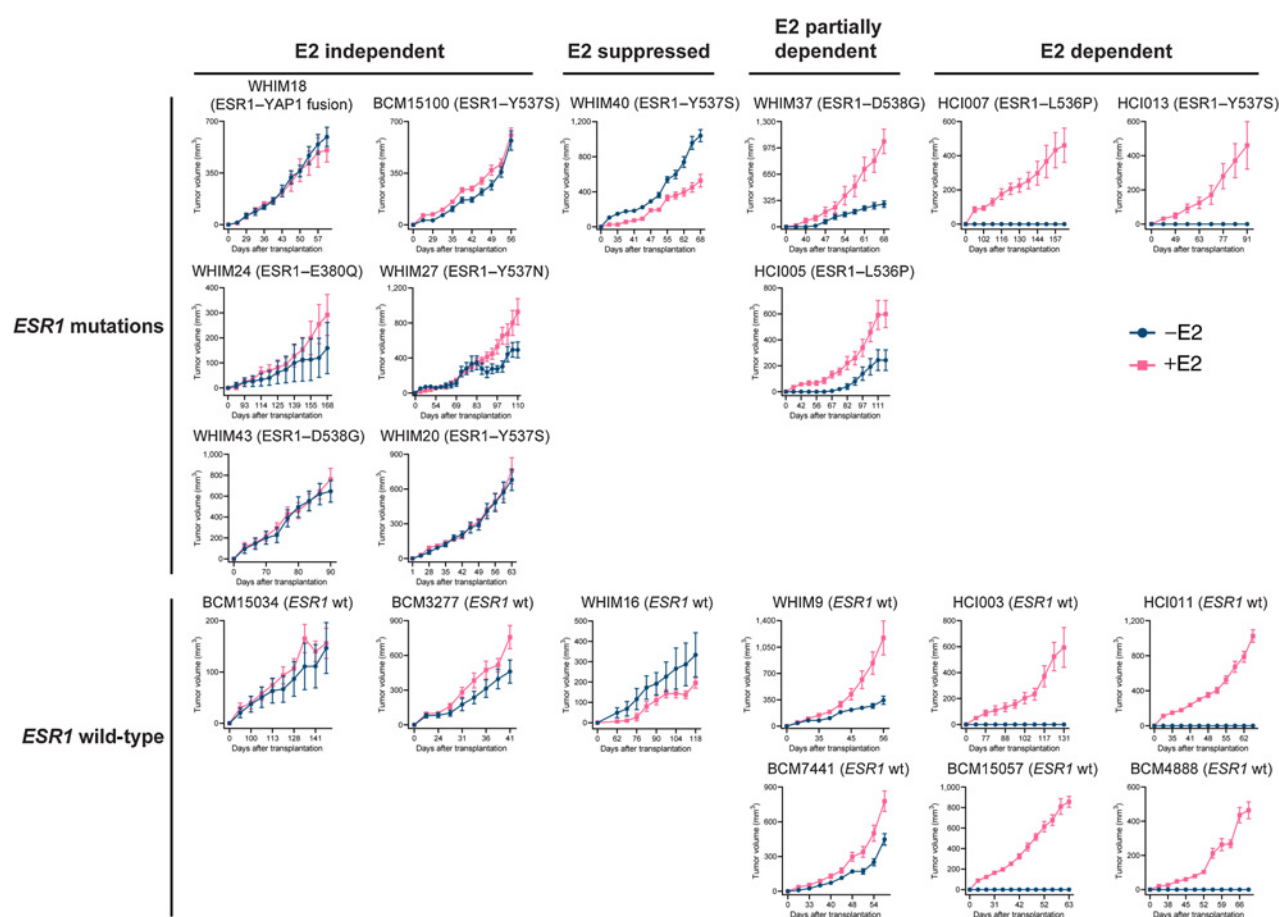


Figure 6.

The growth of 20 ER⁺ PDX tumors in xenografted mice in the absence and presence of E2. Volumes of 20 ER⁺ PDX tumors were measured in ovariectomized SCID/beige mice supplemented with or without 8 µg/mL E2 in the drinking water (mean ± SEM; $n = 7-16$ per PDX line per arm). PDX tumors were categorized based on *ESR1* status (mutations listed or wild-type, wt) and E2 dependency for growth (E2 independent, E2 suppressed, E2 partially dependent, and E2 dependent).

Y537S *ESR1* mutation but remained E2 dependent as previously reported by Welm and colleagues (Fig. 6; ref. 28). Similarly, HCI007 harbors an *ESR1* L536P mutation, but also grew in an E2-dependent manner. These tumors have lower MOTERA scores but still above the training set defined cutoff. Presumably in these examples, *ESR1*-WT is functionally dominant over the LBD-mutant ER α , although the mechanism remains obscure. Under -E2 conditions, the MOTERA signature successfully distinguished between ET-resistant tumors driven by mutant or translocated *ESR1* proteins from *ESR1*-WT PDXs, with an accuracy of 95.0% (specificity, 88.9%; sensitivity, 100%; Fig. 7B). Although the MOTERA transcriptional signature was largely composed of estrogen response genes, expression levels were not affected by E2 supplementation to the WHIM18 *ESR1*-YAP1 expressing PDX or other PDXs expressing *ESR1* LBD point mutations, underscoring sensitivity for the activated *ESR1*-mutant/translocated protein state (Fig. 7A and B). Upon E2 treatment, the MOTERA scores of *ESR1*-WT bearing PDX lines still remained significantly lower than those of *ESR1* mutated tumors, although in several cases, expression levels rose above the cutoff established in E2-deprived conditions (Fig. 7B).

An independent RNA-seq data set of 55 ER⁺ mRNA-positive MBC cases from the MET500 study (9) was used to further evaluate the performance of the MOTERA gene signature in tumor samples.

Signature scores were significantly elevated in tumors expressing *ESR1* LBD point mutations, such as Y537S and D538G, versus *ESR1*-WT samples (Fig. 7C). Two *ESR1* fusions that were functionally studied in Fig. 2 (*ESR1*-e6>ARNT2-e18 and *ESR1*-e6>ARID1B) were both originally identified from the MET500 study. As expected, the *ESR1*-e6>ARNT2-e18 fusion drove a high MOTERA score in the sample in which it was identified. Against predictions, the functionally inactive *ESR1*-e6>ARID1B fusion also had a positive MOTERA signature score. However, this patient sample also harbored an *ESR1*-D538G LBD mutation, likely explaining the discordance. In terms of performance, the MOTERA signature score significantly distinguished active *ESR1* mutations (Y537S, D538G, and Y537C point mutations and the *ESR1*-e6>ARNT2-e18 fusion) from WT *ESR1*, with a sensitivity of 92.9% and a specificity of 78.0% for an AUC of 88.7% (95% confidence interval, 80.0%–97.3%; Fig. 7D).

Discussion

The data presented herein clearly demonstrate that most in-frame *ESR1*-e6 fusion proteins derived from interchromosomal translocations are drivers of ET resistance. Hitherto the clinical importance of *ESR1* gene translocation has been underappreciated because the diversity of C-terminal partner genes creates a considerable diagnostic

challenge. Fluorescence *in situ* hybridization (FISH) and PCR approaches that require the identification of both partners in a gene fusion event are not applicable. Even in the case of *ESR1-e6>ARNT2*, where we identified two examples, the *ARNT2* exons present at the fusion junctions were different. Break-apart FISH probes can be considered in a setting where only one partner in the fusion is known. However, this approach does not identify the unknown 3' partner gene or the reading frame, which is critical because inactive out-of-frame fusions are common (Supplementary Table S1). RNA-seq is clearly an applicable unbiased discovery approach, but sensitive detection requires the identification of a sufficient number of fusion junction reads to confidently diagnose the presence of an in-frame translocation. When RNA-seq coverage is low, or the RNA is of low quality, fusion junction sequences could easily remain undetected.

Adding to the difficulty of understanding the clinical significance of *ESR1* gene fusions is the fact that only a subset of *ESR1* fusion proteins are active, and therefore clinically actionable. Consistent rules to diagnose whether a fusion is active based on the known functions of the C-terminal fusion partners proved hard to define. Although *ESR1-e6* fusions with *YAP1*, *SOX9*, *ARNT2*, *LPP*, and *NCOA1* are all known positive regulators of transcription and produce active fusion proteins, our analysis of the *ESR1-e6>TCF12* fusion protein produced an interesting exception. *TCF12* encodes a bHLH E-box TF, and its two TADs (29) are present in the fusion. Nonetheless, the synthetic *ESR1-e6>TCF12* cDNA was inactive in both T47D and MCF7 cells. We cannot exclude the possibility that this particular fusion is only active in the context of the cancer in which it evolved, i.e., the indicator cell lines we used lack the requisite coactivators. If truly inactive, however, the *ESR1-e6>TCF12* fusion event raises the question of how this example could have been selected during clonal evolution. A potential explanation is provided by the *ESR1-e6>ARID1B* fusion protein, which is transcriptionally inactive with a 3' partner gene related to the established tumor suppressor *ARID1A* (30, 31). It has been proposed that *TCF12* encodes a tumor suppressor (29, 32). Thus, selection of transcriptionally inactive *ESR1* fusions could be explained if these fusions inactivate tumor suppressor functions encoded by the 3' partner gene. One could even speculate that these putative *ESR1* tumor suppressor fusion proteins act in a dominant-negative fashion, thereby interrupting the function of the remaining intact *TCF12* or the *ARID1A* activity. Multiple active non-TF/CoA fusions (*PCDH11X*, *DAB2*, *CLINT1*, *GRIP1*, and *TNRC6B*) dramatically add to the complex landscape of *ESR1* fusion genes. The activity of these fusions cannot, by definition, be predicted from an understanding of the normal function of each 3' partner gene involved because none are known to be a TF or CoA, and the WT protein is not nuclear localized. Presumably the fusion partners have diverse protein-protein interaction domains that are subverted for the purposes of activating gene transcription in the context of a pathologic fusion with *ESR1*. These questions must be addressed in follow-up mechanistic studies.

One diagnostic approach after the detection of an in-frame *ESR1* fusion gene would be to test the newly identified example *in vitro*. However, this is inefficient for clinical care and may not always produce an accurate result. These concerns stimulated the development of the MOTERA gene signature to screen for tumors driven by the diverse somatic events that activate *ESR1* through the presence of a diagnostic gene signature. In a setting where an *ESR1*-activating mutation has already been identified, the MOTERA signature could be used to confirm whether the mutant *ESR1* gene is indeed driving ET

resistance. However, the MOTERA signature is likely to be of most value in the setting where a canonical *ESR1* LBD point mutation has not been detected. Here, a high MOTERA score would warrant further investigation to detect a functional in-frame *ESR1-e6* fusion. Reflex diagnostic approaches for these cases could include unbiased RNA-seq, *ESR1*-specific 3' rapid amplification of cDNA ends (3'-RACE) or break-apart *ESR1* FISH. Although break-apart FISH would not identify the C-terminal partner, its presence has already been signaled by a positive MOTERA score implying the unknown partner in the chimera is transcriptionally active.

Analysis of the MET500 data indicates that MBC with high MOTERA scores but without an *ESR1* point mutation detected by genome sequencing or translocation detected by RNA-seq are not infrequent (Fig. 7C). Possibilities for these cases include: (i) the RNA-seq result was false-negative for the presence of an active *ESR1* fusion; (ii) the exome sequencing was a false-negative for the presence of an *ESR1* mutation; (iii) the MOTERA score was a false-positive that reflects WT ER α activity because the sample was taken when the patient was not taking ET and the tumor was still E2 dependent; and (iv) some WT ER α MBC persist by expressing a similar MOTERA signature that might be driven by other mechanisms, like TFs other than ER α . The prospective evaluation of the MOTERA signature is therefore the next phase of our investigation.

An important focus for future studies will be to determine the clinical characteristics of *ESR1* fusion-driven tumors. Of particular interest is an examination of the metastatic spread associated with tumors expressing *ESR1* gene fusions, as in mouse xenograft systems, active *ESR1* fusions drive lung metastasis (7). Distinct from WT ER α , active *ESR1* fusions strongly induce EMT-related genes, which we functionally annotated using motility and invasion assays. This property differentiates the MOTERA signature from other gene sets that measure activity of the ER α pathway, such as the Hallmark early/late estrogen response gene set. Consistent with this, the MOTERA scores of *ESR1*-mutated PDX tumors were still significantly higher than those of *ESR1*-WT-bearing lines that received E2 treatment. Interestingly, EMT-related gene expression is elevated during mammary gland development as the nascent ducts invade the mammary fat pad, and then EMT gene expression is reduced after puberty (33, 34). Thus, active *ESR1-e6* fusion proteins may be reactivating a developmental EMT program that is usually silenced in mature breast epithelial cells. Specific examples of *ESR1* fusion-induced genes in the MOTERA signature that are related to metastasis include *SGK1*, which encodes serum- and glucocorticoid-inducible kinase 1 and promotes breast cancer bone metastasis (35). *VCAN* encodes versican, whose expression is significantly correlated with metastasis and poor overall survival (36). *GJA1* encodes connexin-43, a gap junction protein that mediates tumor cell migration and invasion (37, 38). *GFR1* encodes GFR α that acts as a coreceptor in conjunction with the RET receptor, and activation of GFR α -RET signaling by binding the glial-derived neurotrophic factor (GDNF) ligand leads to ER α serine phosphorylation and enhanced transcriptional activity (39).

At least one *ESR1* fusion partner gene described herein has been observed in other settings. Gene fusions involving *LPP*, the gene encoding the Lipoma Preferred Partner protein, such as a recurrent *HMG2-LPP* fusion have been found in multiple tumors, including lipoma (40), pulmonary chondroid hamartomas (41), and chondromas (42). In leukemia, an *MLL-LPP* fusion has been identified (43). Similar to the *ESR1-e6>LPP* fusion, these fusions preserve the three C-terminal LIM domains encoded by the *LPP* gene, which serve as the

binding site for the ETS domain TF PEA3 and contain coactivator activity (44). It is therefore likely that in larger studies, some *ESR1* gene fusions will be observed to be recurrent, making the diagnosis of some *ESR1* translocations easier.

In conclusion, *ESR1-e6* gene fusions are part of the spectrum of the somatic mutations that constitutively activate ESR1 proteins in advanced ER⁺ breast cancer to drive poor outcomes. The MOTERA signature should be useful to answer the question how common these events are, because it will focus sensitive fusion detection approaches on cases where there is transcriptional evidence for an activating *ESR1* fusion (or mutation) that has not been diagnosed yet. Once the clinical significance of *ESR1* gene fusions becomes more widely recognized and the diagnostic approach becomes more efficient, specific treatment approaches for tumors expressing active ESR1 fusion proteins can be developed.

Authors' Disclosures

X. Gou reports grants from Cancer Prevention and Research Institute of Texas (CPRIT) Training Grant during the conduct of the study; in addition, X. Gou has a patent for 24-gene diagnostic signature pending to BCM. L.E. Dobrolecki reports personal fees from StemMed, Ltd., outside the submitted work. M.T. Lewis reports grants from NCI and CPRIT during the conduct of the study and other support from StemMed Ltd and Tvardi Therapeutics Inc. outside the submitted work. A.L. Welm reports other support from Thunder Biotech, Modulus Therapeutics, Inc., and J. Michael Bishop Institute for Cancer Research outside the submitted work; and A.L. Welm may receive tangible property royalties if University of Utah chooses to license HCI PDX models to for-profit companies. S. Li reports grants, personal fees, and nonfinancial support from NIH 5U54CA224083 during the conduct of the study and personal fees from Envigo outside the submitted work. C.E. Foulds reports grants from Adrienne Helis Malvin Medical Research Foundation during the conduct of the study; other support from Coactigon, Inc., outside the submitted work; in addition, C.E. Foulds has a patent for 24-gene diagnostic signature pending to BCM. M.J. Ellis reports grants from NCI, Susan G. Komen Foundation, DOD Breast Cancer Research Program, McNair Medical Institute at The Robert and Janice McNair Foundation, CPRIT, and Adrienne Helis Malvin Medical Research Foundation during the conduct of the study; personal fees from Lilly, Novartis, AstraZeneca, Pfizer, Veracyte, Bioclassifier, and Washington University in St Louis outside the submitted work; in addition, M.J. Ellis has a patent for MOTERA signature pending. No disclosures were reported by the other authors.

References

- Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. *Annu Rev Med* 2011;62:233–47.
- Ma CX, Reinert T, Chmielewska I, Ellis MJ. Mechanisms of aromatase inhibitor resistance. *Nat Rev Cancer* 2015;15:261–75.
- Dustin D, Gu G, Fuqua SAW. ESR1 mutations in breast cancer. *Cancer* 2019;125:3714–28.
- Fribbens C, O'Leary B, Kilburn L, Hrebien S, Garcia-Murillas I, Beaney M, et al. Plasma ESR1 mutations and the treatment of estrogen receptor-positive advanced breast cancer. *J Clin Oncol* 2016;34:2961–8.
- Spoerke JM, Gendreau S, Walter K, Qiu J, Wilson TR, Savage H, et al. Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant. *Nat Commun* 2016;7:11579.
- Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep* 2013;4:1116–30.
- Lei JT, Shao J, Zhang J, Iglesia M, Chan DW, Cao J, et al. Functional annotation of *esr1* gene fusions in estrogen receptor-positive breast cancer. *Cell Rep* 2018;24:1434–44.
- Hartmaier RJ, Trabucco SE, Priedigkeit N, Chung JH, Parachoniak CA, Borre V, et al. Recurrent hyperactive ESR1 fusion proteins in endocrine therapy-resistant breast cancer. *Ann Oncol* 2018;29:872–80.
- Robinson DR, Wu YM, Lonigro RJ, Vats P, Cobain E, Everett J, et al. Integrative clinical genomics of metastatic cancer. *Nature* 2017;548:297–303.
- Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, et al. Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. *Cell* 2012;149:307–21.
- Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 2011;12:323.
- Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;9:357–9.
- Zhang X, Claerhout S, Prat A, Dobrolecki LE, Petrovic I, Lai Q, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. *Cancer Res* 2013;73:4885–97.
- DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nat Med* 2011;17:1514–20.
- Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011;12:77.
- Foulds CE, Feng Q, Ding C, Bailey S, Hunsaker TL, Malovannaya A, et al. Proteomic analysis of coregulators bound to ERalpha on DNA and nucleosomes reveals coregulator dynamics. *Mol Cell* 2013;51:185–99.
- Gates LA, Gu G, Chen Y, Rohira AD, Lei JT, Hamilton RA, et al. Proteomic profiling identifies key coactivators utilized by mutant ERalpha proteins as potential new therapeutic targets. *Oncogene* 2018;37:4581–98.

Authors' Contributions

X. Gou: Conceptualization, data curation, software, formal analysis, validation, investigation, visualization, methodology, writing—original draft, writing—review and editing. M. Anurag: Data curation, software, formal analysis, and methodology. J.T. Lei: Investigation and methodology. B. Kim: Data curation and investigation. P. Singh: Investigation. S. Seker: Investigation. D. Fandino: Investigation. A. Han: Investigation. S. Rehman: Investigation. J. Hu: Investigation. V. Korchina: Investigation. H. Doddapaneni: Investigation. L.E. Dobrolecki: Resources. N. Mitsiades: Funding acquisition. M.T. Lewis: Resources and funding acquisition. A.L. Welm: Resources and funding acquisition. S. Li: Resources. A.V. Lee: Resources. D.R. Robinson: Resources. C.E. Foulds: Conceptualization, resources, data curation, investigation, methodology, writing—review and editing. M.J. Ellis: Conceptualization, resources, supervision, funding acquisition, project administration, writing—review and editing.

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18. Bagchi MK, Tsai SY, Tsai MJ, O'Malley BW. Identification of a functional intermediate in receptor activation in progesterone-dependent cell-free transcription. *Nature* 1990;345:547–50.
19. UniProt C. UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res* 2021;49:D480–D9.
20. LaRese TP, Rheaume BA, Abraham R, Eipper BA, Mains RE. Sex-specific gene expression in the mouse nucleus accumbens before and after cocaine exposure. *J Endocr Soc* 2019;3:468–87.
21. Howell A, Osborne CK, Morris C, Wakeling AE. ICI 182,780 (Faslodex): development of a novel, "pure" antiestrogen. *Cancer* 2000;89:817–25.
22. Carroll JS, Brown M. Estrogen receptor target gene: an evolving concept. *Mol Endocrinol* 2006;20:1707–14.
23. Ye X, Tam WL, Shibue T, Kaygusuz Y, Reinhardt F, Eaton N, et al. Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. *Nature* 2015;525:256–60.
24. Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* 2000;2:76–83.
25. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545–50.
26. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdottir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics* 2011;27:1739–40.
27. Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Shale C, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature* 2019;575:210–6.
28. Sikora MJ, Cooper KL, Bahreini A, Luthra S, Wang G, Chandran UR, et al. Invasive lobular carcinoma cell lines are characterized by unique estrogen-mediated gene expression patterns and altered tamoxifen response. *Cancer Res* 2014;74:1463–74.
29. Labreche K, Simeonova I, Kamoun A, Gleize V, Chubb D, Letouze E, et al. TCF12 is mutated in anaplastic oligodendroglioma. *Nat Commun* 2015;6:7207.
30. Nagl NG, Jr., Patsialou A, Haines DS, Dallas PB, Beck GR, Jr., Moran E. The p270 (ARID1A/SMARCF1) subunit of mammalian SWI/SNF-related complexes is essential for normal cell cycle arrest. *Cancer Res* 2005;65:9236–44.
31. Shain AH, Giacomini CP, Matsukuma K, Karikari CA, Bashyam MD, Hidalgo M, et al. Convergent structural alterations define SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. *Proc Natl Acad Sci U S A* 2012;109: E252–9.
32. Chen QB, Liang YK, Zhang YQ, Jiang MY, Han ZD, Liang YX, et al. Decreased expression of TCF12 contributes to progression and predicts biochemical recurrence in patients with prostate cancer. *Tumour Biol* 2017;39:1010428317703924.
33. Sikandar SS, Kuo AH, Kalisky T, Cai S, Zabala M, Hsieh RW, et al. Role of epithelial to mesenchymal transition associated genes in mammary gland regeneration and breast tumorigenesis. *Nat Commun* 2017;8:1669.
34. Foubert E, De Craene B, Berx G. Key signalling nodes in mammary gland development and cancer. The Snail1-Twist1 conspiracy in malignant breast cancer progression. *Breast Cancer Res* 2010;12:206.
35. Zhang Z, Xu Q, Song C, Mi B, Zhang H, Kang H, et al. Serum- and glucocorticoid-inducible kinase 1 is essential for osteoclastogenesis and promotes breast cancer bone metastasis. *Mol Cancer Ther* 2020;19:650–60.
36. Mitsui Y, Shiina H, Kato T, Maekawa S, Hashimoto Y, Shiina M, et al. Versican promotes tumor progression, metastasis and predicts poor prognosis in renal carcinoma. *Mol Cancer Res* 2017;15:884–95.
37. Pollmann MA, Shao Q, Laird DW, Sandig M. Connexin 43 mediated gap junctional communication enhances breast tumor cell diapedesis in culture. *Breast Cancer Res* 2005;7:R522–34.
38. Kotini M, Mayor R. Connexins in migration during development and cancer. *Dev Biol* 2015;401:143–51.
39. Plaza-Menacho I, Morandi A, Robertson D, Pancholi S, Drury S, Dowsett M, et al. Targeting the receptor tyrosine kinase RET sensitizes breast cancer cells to tamoxifen treatment and reveals a role for RET in endocrine resistance. *Oncogene* 2010;29:4648–57.
40. Crombez KR, Vanoirbeek EM, Van de Ven WJ, Petit MM. Transactivation functions of the tumor-specific HMGA2/LPP fusion protein are augmented by wild-type HMGA2. *Mol Cancer Res* 2005;3:63–70.
41. Rogalla P, Lemke I, Kazmierczak B, Bullerdiek J. An identical HMGIC-LPP fusion transcript is consistently expressed in pulmonary chondroid hamartomas with t(3;12)(q27–28;q14–15). *Genes Chromosomes Cancer* 2000;29: 363–6.
42. Dahlen A, Mertens F, Rydholm A, Brosjo O, Wejde J, Mandahl N, et al. Fusion, disruption, and expression of HMGA2 in bone and soft tissue chondromas. *Mod Pathol* 2003;16:1132–40.
43. Daheron L, Veinstein A, Brizard F, Drabkin H, Lacotte L, Guilhot F, et al. Human LPP gene is fused to MLL in a secondary acute leukemia with a t(3;11)(q28;q23). *Genes Chromosomes Cancer* 2001;31:382–9.
44. Guo B, Sallis RE, Greenall A, Petit MM, Jansen E, Young L, et al. The LIM domain protein LPP is a coactivator for the ETS domain transcription factor PEA3. *Mol Cell Biol* 2006;26:4529–38.

Supplementary Information

Transcriptional reprogramming differentiates active from inactive ESR1 fusions in endocrine therapy-refractory metastatic breast cancer

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List of Supplementary Material

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4) Supplemental References

1) Supplementary Methods

Cell Culture

All cell lines were obtained from the Tissue Culture Core at BCM in 2017, which originally came from ATCC. Cells were cultured at 37°C in 5% CO₂ and were examined for mycoplasma every 6 months. In all experiments, the cells were no longer used after passage 20. T47D (ATCC Cat# HTB-133, RRID: CVCL_0553) and MCF7 (ATCC Cat# HTB-22, RRID: CVCL_0031) cells were grown in RPMI1640 with L-Glutamine (Corning, cat#10-040-CV) supplemented with 10% FBS (Sigma-Aldrich, cat#F8067), glucose to 4.5 g/L (Sigma, cat# G5767), 10 mM HEPES (GenDEPOT, cat# CA011), 1 mM sodium pyruvate (GenDEPOT, cat# CA017), and 50 µg/mL gentamycin (GenDEPOT, cat# CR003-001). For hormone deprivation, cells were plated in culture media overnight, washed with Dulbecco's PBS, and maintained in phenol red-free, RPMI media (Fisher Scientific, cat#11835) containing charcoal stripped serum (Sigma, cat#F6765) as supplemented as above (CSS media). CSS media was changed every 2-3 days for 1-2 weeks. HEK293T (ATCC Cat# CRL-3216, RRID: CVCL_0063) cells were grown in DMEM with high-glucose (4.5 g/L), L-glutamine and sodium pyruvate (Mediatech, cat#10-013-CV) supplemented with 10% FBS (Sigma, cat#F8067) and 1% penicillin-streptomycin (Sigma, cat#P4333).

Subcloning of ESR1 fusions into a lentiviral expression vector

HA-tagged cDNAs encoding ESR1-e6>DAB2, ESR1-e6>GYG1, ESR1-e6>SOX9, ESR1-e6>ARNT2-e18, ESR1-e6>PCMT1 and ESR1-e6>ARID1B were subcloned from a pcDNA 3.1(+) vector (Gene Universal) into pCDH-CMV-MCS-EF1a-Puro lentiviral vector (System Biosciences, cat#CD510B-1) by restriction digestion and ligation with T4 DNA ligase, followed by DNA sequencing for verification. The synthesis, subcloning, and sequence verification of additional *ESR1* fusion cDNA constructs (ESR1-e6>ARNT2-e2, ESR1-e6>LPP, ESR1-e6>NCOA1, ESR1-e6>TCF12, ESR1-e6>CLINT1, ESR1-e6>GRIP1 and ESR1-e6>TNRC6B) into pCDH-CMV-MCS-EF1a-Puro lentiviral vector was performed by Gene Universal.

Generation of lentiviral stable ESR1 fusion expressing cell lines

The culture media containing lentiviral particles were collected after 48 hours, filtered using 0.45 µm pore filter (Millipore), and added to T47D (MOI=1) and MCF7 (MOI=2) cells in the presence of 8 µg/ml polybrene (Millipore, cat#TR-1003-G).

Immunoblotting and immunoprecipitation

Cells were harvested by scraping in cold PBS. Cell pellets were resuspended in either RIPA lysis buffer (Thermo Fisher Scientific, cat#89900) or MIB lysis buffer (50 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA with 0.5% Triton X-100) supplemented with 1x protease inhibitors and 1x phosphatase inhibitors, and lysed on ice for 30 min or sonicated for 2 minutes on a Corvaris sonicator, respectively. Cell lysates were clarified by centrifugation at 20,000x g for 30 minutes at 4°C. Protein concentrations were determined by Bradford assay (Bio-Rad). Proteins (20 µg) were mixed with LDS Sample Buffer (Invitrogen, cat#NP0007), separated on 4%–12% gradient gels (Thermo Fisher Scientific), transferred to nitrocellulose membrane. Primary antibodies used for immunoblotting were as follows: N-terminal ERα (Millipore, Cat#04-820; RRID: AB_1587018, 1:1000), C-terminal ERα (Cell Signaling Technology Cat#8644, RRID: AB_2617128, 1:1000), Snail (Cell Signaling Technology, Cat#3879; RRID: AB_2255011, 1:1000), E-Cadherin (Cell Signaling Technology, Cat#14472; RRID: AB_2728770, 1:1000), GAPDH (Santa Cruz Biotechnology Cat# sc-47724, RRID: AB_627678, 1:5000) and DNA-PKcs (Santa Cruz Biotechnology Cat# sc-5282, RRID: AB_2172848, 1:1000). The following HRP-conjugated secondary antibodies (anti-rabbit IgG, Cell Signaling Technology Cat# 7074, RRID: AB_2099233, 1:10,000) and anti-mouse IgG (Cell Signaling Technology Cat# 7076, RRID: AB_330924, 1:10,000) were employed and membranes were developed using ECL Detection Reagent (Fisher Scientific, cat#RPN2235) with visualization on a Bio-Rad Imaging System.

Hormone-deprived stable T47D cells were treated with or without 100 nM E2 for 45 minutes at 37°C, harvested and lysed in IP lysis buffer (Thermo Fisher Scientific, cat#87787) containing 1x protease inhibitors and 1x phosphatase inhibitors. Whole cell lysates (2 mg) were immunoprecipitated with 2 µg anti-HA tag antibody (Santa Cruz Biotechnology, mouse, Cat#sc-7392; RRID: AB_627809), or mouse

IgG Control (Cell Signaling Technology Cat# 61656, RRID: AB_2799613) overnight at 4°C with rotation, followed by incubation of protein A magnetic beads (Bio-Rad, cat# 1614013) for 1h at 4°C and washing with IP lysis buffer for 3 times.

Cell growth and motility assays

Hormone-deprived stable cells were plated in 96-well plates (3,000 cells/well). One day after plating, cells were treated with or without 100 nM fulvestrant in the presence or absence of 10 nM E2. CSS media containing drugs were changed every 2-3 days. Cell growth was quantified using an alamarBlue reagent (resazurin sodium salt, Sigma, R7017, 0.25 mg/ml) after 7-10 days of treatment, and the plates were read by taking the ratio of 540 nm ($\lambda_{\text{Excitation}}$)/ 590 nm ($\lambda_{\text{Emission}}$) with a fluorescent microplate reader (BMG LABTECH).

Cell motility was detected using a scratch wound assay. Hormone-deprived cells were plated at 70,000 cell/well in a 96-well ImageLock plate overnight, and then pre-treated with mitomycin C before being subjected to wounding by a WoundMaker (Essen BioScience). CSS media containing mitomycin C was changed every 24 hours.

RNA-Seq and analysis

The GARP core made mRNA libraries from T47D RNA samples using the Illumina TruSeq Stranded mRNA library preparation protocol (p/n 1000000040498 v00, Oct 2017) along with ThermoFisher's ERCC RNA Spike-In Control Mixes Protocol (p/n 4455352, rev. D) using 250 ng of starting total RNA. The resulting libraries were quantitated using the NanoDrop spectrophotometer and fragment size assessed with the Agilent Bioanalyzer. A qPCR quantitation was performed on the libraries to determine the concentration of adapter ligated fragments using the Applied Biosystems ViiA7 Real-Time PCR System and a KAPA Library Quant Kit (p/n KK4824). All samples were pooled equimolarly and re-quantitated by qPCR, and also re-assessed on the Bioanalyzer. Using the concentration from the ViiA7TM qPCR machine above, 150 pM of equimolarly pooled library was loaded onto one lane of the NovaSeq S1 v1.5 flowcell (Illumina p/n 20028318) following the XP v1.5 Workflow protocol (Illumina kit

p/n 20043130) and amplified by exclusion amplification onto a nanowell-designed, patterned flowcell using the Illumina NovaSeq 6000 sequencing instrument. PhiX Control v3 adapter-ligated library (Illumina p/n FC-110-3001) was spiked-in at 1% by weight to ensure balanced diversity and to monitor clustering and sequencing performance. A paired-end 100 bp cycle run was used to sequence the flowcell on a NovaSeq 6000 Sequencing System. An average of 25.7 million (M) read pairs per sample was sequenced.

For RNA-Seq on isolated ER+ PDX tumors, RNA-Seq was performed at the Human Genome Sequencing Center at BCM as follows. Strand-specific, poly-A+ enriched RNA-seq libraries for sequencing on the Illumina platform were prepared as described (1). Briefly, poly-A+ mRNA was extracted from 1 µg total RNA, followed by fragmentation and first strand cDNA synthesis. The resultant cDNA was end-repaired, A-tailed and ligated with Illumina dual barcode adapters. Libraries were sequenced in groups of 70 on NovaSeq 6000 instrument using the S4 reagent kit (300 cycles) to generate 2 x 150 bp paired-end reads. Between 50 and 85M total reads were generated. The average strand-specificity and rRNA rate was 98% and 1.5%, respectively. The transcripts for 17,000 to 27,087 genes were detected in these samples.

Unsupervised hierarchical clustering was performed using a metric of Euclidean distance and the average linkage method. Differential expressed genes were analyzed using log(2)-transformed, upper quartile-normalized counts per million values with “edgeR” package in R (2) comparing active *ESR1* fusions to inactive fusions and controls.

Whole exome sequencing (WES) and ESR1 gene variant analysis

WES data was generated by the Human Genome Sequencing Center at BCM using the Illumina platform. For this, paired-end libraries were constructed as described (3) with the following modifications. Samples were barcoded at the ligation step using Illumina unique dual barcodes adapters (cat# 20022370) and were amplified 6-8 cycles using the Library Amplification Ready-mix containing KAPA HiFi DNA Polymerase (Kapa Biosystems, Inc). For capture enrichment, libraries were

pooled in equimolar ratios in groups of 10 and were hybridized in solution to the HGSC VCRome 2.1 design. To this design, exome coverage across >3,500 clinically relevant genes that previously were covered below 20X (~2.72 Mb) was supplemented. Enriched libraries were sequenced on the NovaSeq 6000 instrument using the S4 reagent kit (300 cycles) to generate 2 x 150 bp paired-end reads. On average, 11.2 Gb of unique sequence data was generated with 95.3% of the bases in the exome design covered to 20x read depth or greater.

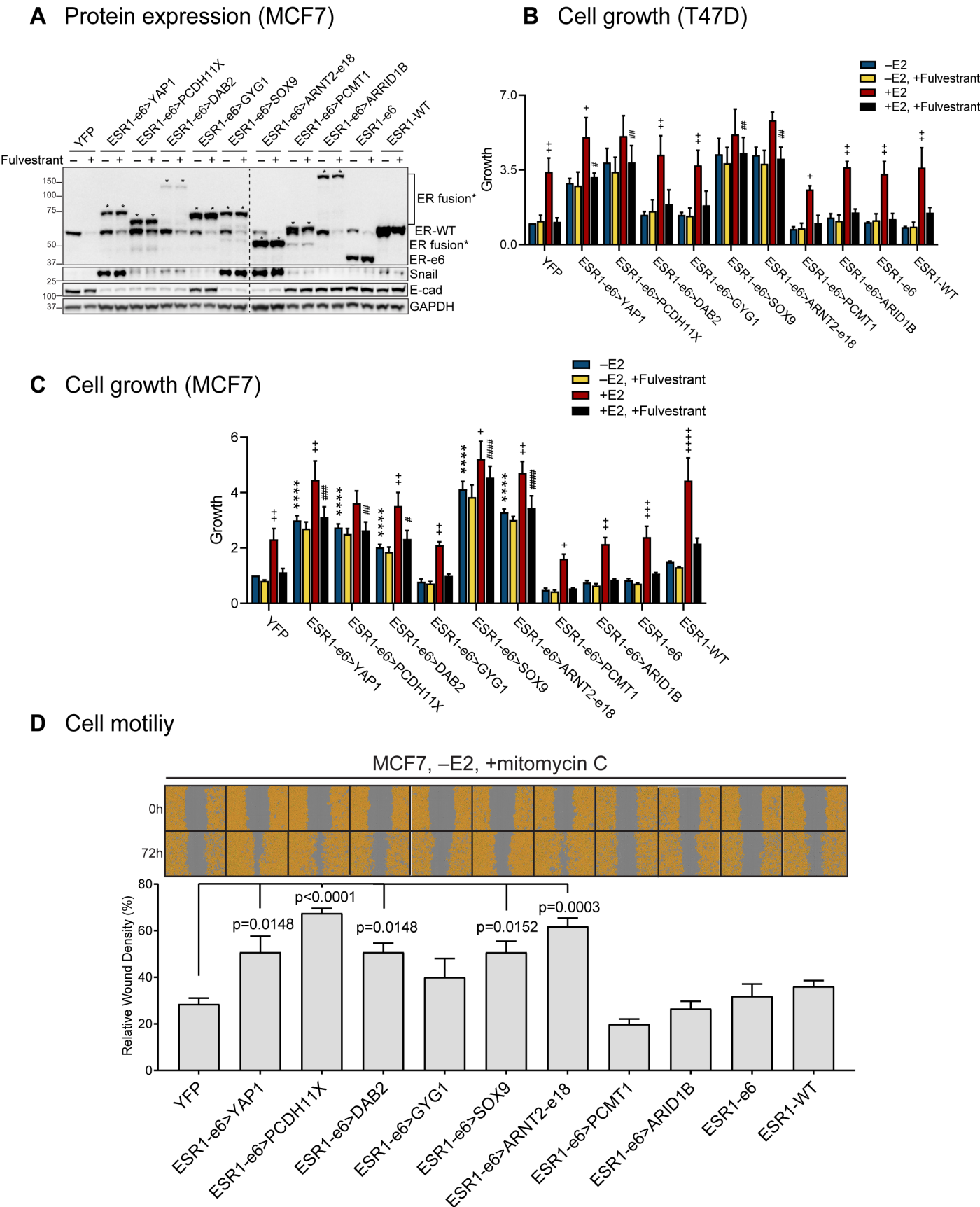
Somatic *ESR1* gene variants were called Strelka2, Mutect2, and CARNAC (v 0.2.5b9). Variants reported by these tools were filtered using GATK VariantFiltration (v 3.8.0) with parameters window 35, cluster 3, FS > 30.0, and QD < 2.0. We kept single nucleotide variants (SNVs) called by any two callers among Strelka2, Mutect2, and CARNAC. We filtered somatic SNVs by a minimal variant allele frequency (VAF) of 0.02. Then we used annovar (v 04.16.2018) to annotate the remaining variants.

Immunofluorescence

Hormone-deprived stable T47D cells were plated onto poly-D-lysine coated coverslips (Fisher) and grown overnight. Cells were fixed with 4% formaldehyde for 20 min at RT followed by permeabilization with 0.2% Triton X-100 for 10 min at RT and then blocked with 10% normal goat serum for 1h. HA-tagged ESR1 fusion proteins were detected with an anti-HA antibody (Cell Signaling Technology Cat# 2367, RRID:AB_10691311, 1:50) overnight at 4°C and then goat anti-mouse IgG secondary antibody (Alexa Fluor 568, Molecular Probes Cat# A-11004, RRID:AB_2534072, 1:1000) for 30 min at RT. Coverslips were mounted onto slides with ProLong Gold Antifade Reagent (Invitrogen). Fluorescence images were taken using a Nikon Eclipse Ti microscope equipped with a CoolSNAP EZ camera (Photometrics Scientific), a Plan Apo 40X/0.95 aperture objective and Nikon NIS elements software.

2) Supplementary Figures

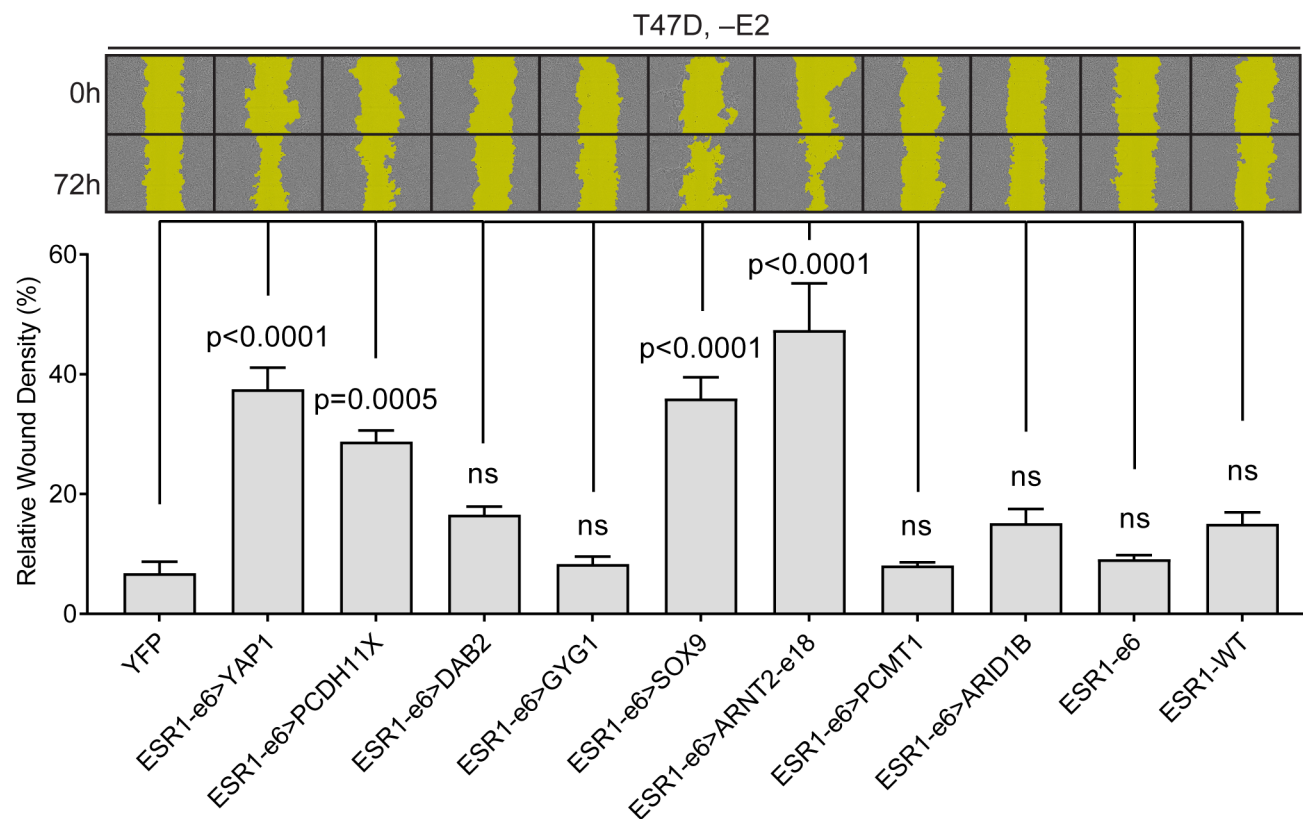
Supplementary Figure 1, related to Figure 2



Supplementary figure 1. In-frame ESR1 fusions drive ET-resistant cell growth and promote hormone-independent cell motility. (A) Immunoblotting of ER α and ESR1 fusion proteins with an N-terminal ER α antibody, Snail or E-cad in hormone-deprived stably expressing MCF7 cells. GAPDH serves as a loading control. Asterisks indicate ESR1 fusion proteins. The dashed line indicates two separate blots that were conducted at the same time. The representative image is from two independent experiments. **(B)** Cell growth was assayed in stably expressing T47D cell controls (YFP, ESR1-e6, or ESR1-WT) or eight ESR1 fusion proteins (mean $\pm$ SEM, n=3). Two-way ANOVA test was used to compare each stable cell after 10 nM estradiol treatment (–E2 vs +E2, *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001). One-way ANOVA followed by Dunnett’s multiple comparisons test was used to compare each ESR1 fusion expressing cell line to YFP control cells in the presence of E2 and fulvestrant (+E2, +Fulvestrant) (#p<0.05, ##p<0.01, ###p<0.001 and ####p<0.0001). **(C)** Cell growth was assayed in MCF7 cells stably expressing various *ESR1* constructs (mean $\pm$ SEM, n=3). One-way ANOVA followed by Dunnett’s multiple comparisons test was used to compare each stable MCF7 cell line to YFP control cells. In the absence of estradiol (–E2), ****p<0.0001. In the presence of E2 and fulvestrant (+E2, +Fulvestrant), #p<0.05, ##p<0.01, ###p<0.001 and ####p<0.0001. Two-way ANOVA was used to compare each stable cell line after 10 nM estradiol treatment (–E2 vs +E2, *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001). **(D)** Cell motility was detected using scratch wound assays in hormone-deprived stably expressing MCF7 cells, treated with 200 ng/ml mitomycin to block proliferation (mean $\pm$ SEM, n=3). One-way ANOVA followed by Dunnett’s multiple comparisons test was used to compare each cell line to YFP control cells. Cells are pseudo-colored orange to aid visualization.

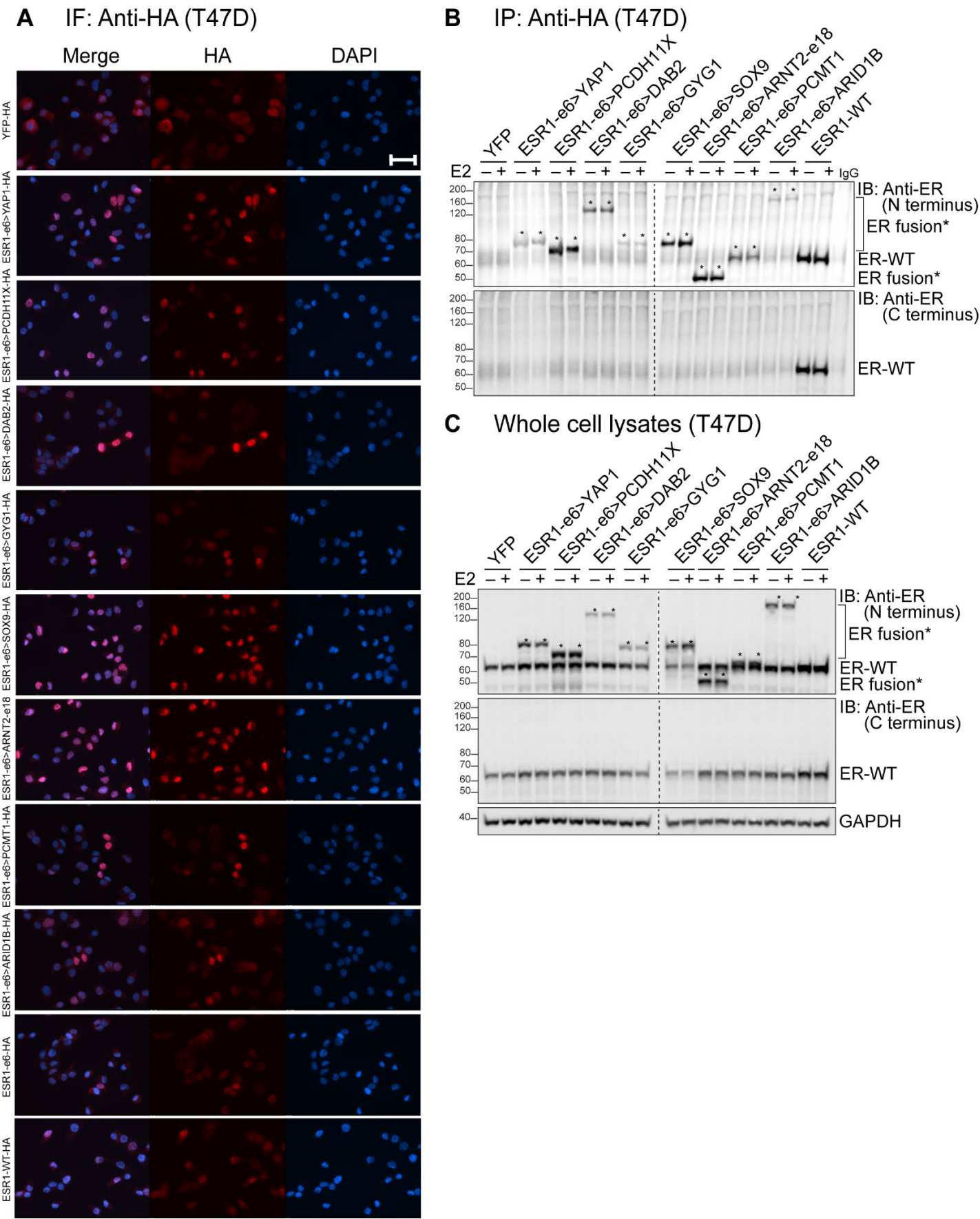
Supplementary Figure 2, related to Figure 2

Cell invasion



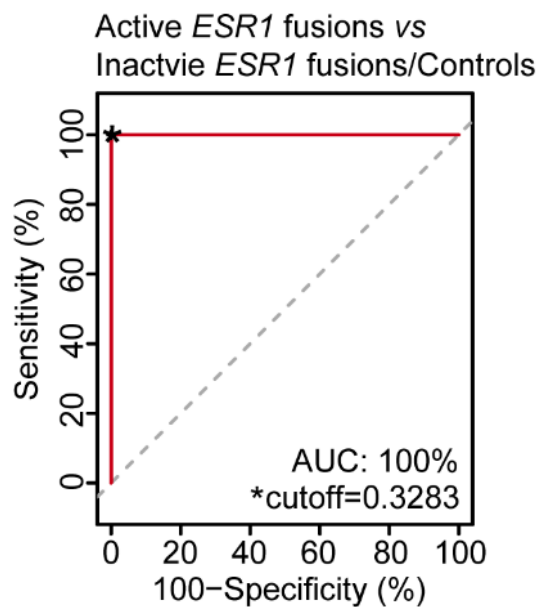
Supplementary figure 2. Active in-frame ESR1 fusions promote hormone-independent cell invasion. Cell invasion was detected in hormone-deprived stably expressing T47D cells, in a similar manner to the scratch wound assay except that cells were first plated on a Matrigel-coated plate, as described in the Methods. *Top panel*, IncuCyte images were recorded. *Bottom panel*, relative wound densities (%) were calculated and plotted (mean $\pm$ SEM, n=3). One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare each cell line to YFP control cells. Wound regions are pseudo-colored green to aid visualization.

Supplementary Figure 3, related to Figure 3



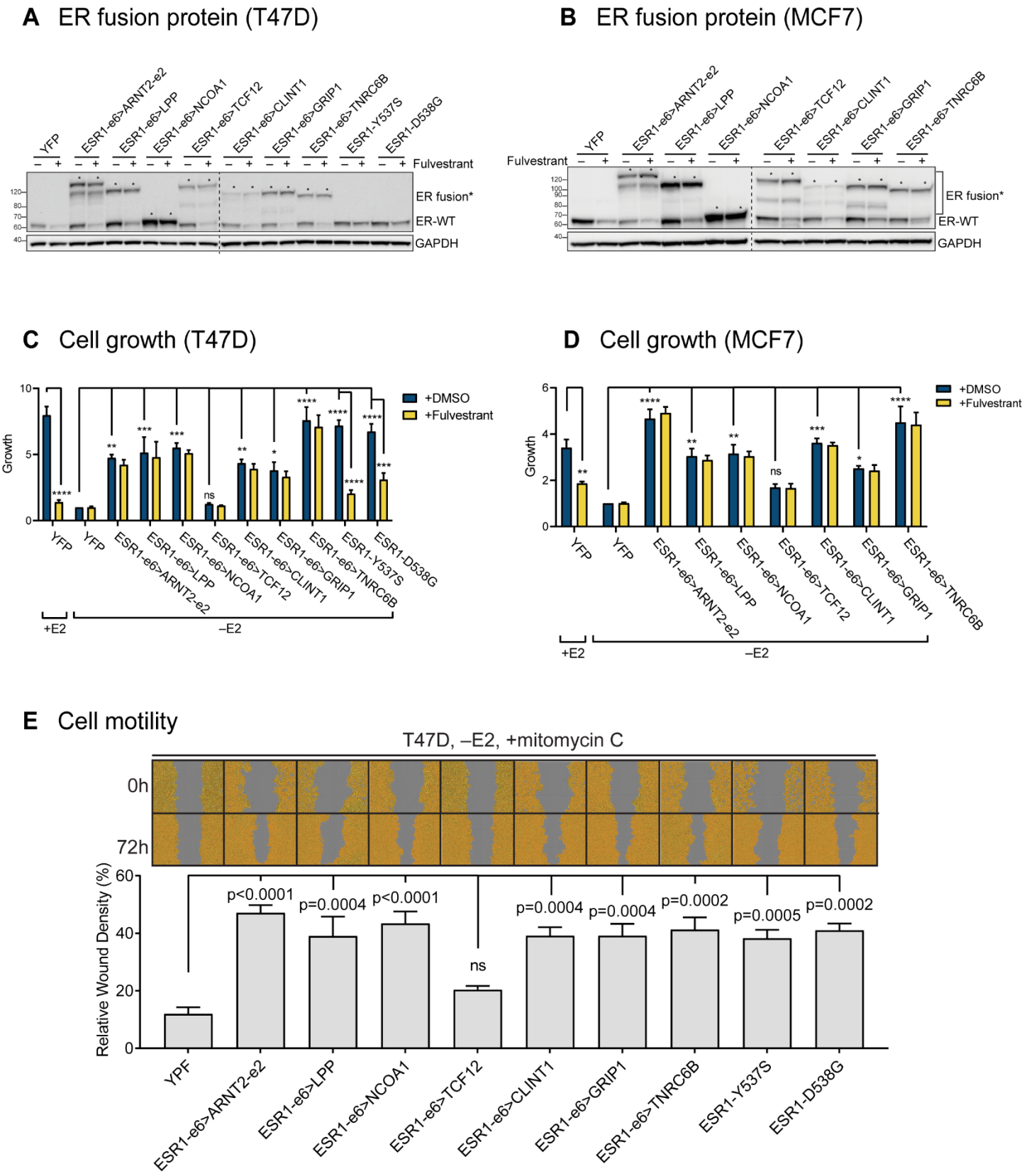
Supplementary figure 3. ESR1 fusion proteins localize to the nucleus and do not form heterodimers with endogenously expressed ER α . **(A)** Immunofluorescence staining with anti-HA tag antibody (red) on hormone-deprived stably expressing T47D cells showed nuclear localization of all eight HA-tagged *ESR1* fusion constructs. The nucleus was labeled with DAPI (blue). Representative images from two independent experiments are shown. Magnification 40X; Scale bar, 50 μ m. **(B)** Hormone-deprived T47D cells stably expressing different *ESR1* constructs were treated with or without 100 nM E2 for 45 min. Cell lysates were then immunoprecipitated with an anti-HA antibody or mouse IgG control and then blotted with a N-terminal ER α antibody. Blotting with a C-terminal ER α antibody only detected ER-WT, and no co-immunoprecipitation with any *ESR1* fusion protein. Representative images are from two independent experiments. The dashed line indicates two separate blots that were conducted at the same time. **(C)** Whole cell lysates (1% inputs for co-IP) were detected by immunoblotting with N- and C-terminal ER α antibodies, along with GAPDH as the loading control.

Supplementary Figure 4, related to Figure 4



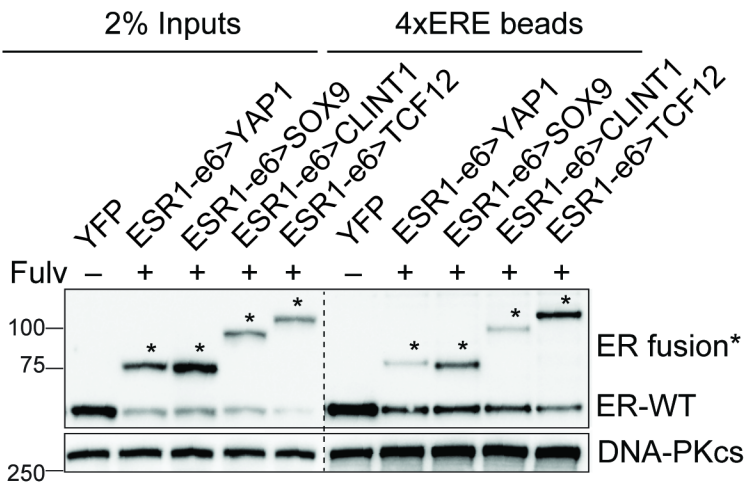
Supplementary figure 4. ROC curve for the 24-gene signature to classify activities of *ESR1* fusions in the original training set. A cutoff of 0.3283 for mean signature score was defined.

Supplementary Figure 5, related to Figure 5



Supplementary figure 5. Additional ESR1 fusions identified in ER+ MBC patients drive ET-resistant growth and promote hormone-independent motility of ER+ breast cancer cells. (A and B) Immunoblotting of ER α and ESR1 fusion proteins with an N-terminal ER α antibody in hormone-deprived stably expressing T47D and MCF7 cells. Asterisks indicate ESR1 fusion proteins. GAPDH serves as a loading control. The dashed line indicates two separate blots that were conducted at the same time. The representative image is from 2-3 independent experiments. **(C and D)** Cell growth was assayed in hormone-deprived cells stably expressing an *ESR1* construct (mean $\pm$ SEM, n=3). One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare data of hormone-deprived *ESR1* fusion expressing cells to YFP control cells in vehicle control (+DMSO) group. Two-way ANOVA followed by Bonferroni's test was used for multiple comparisons for each stable cell line after 100 nM fulvestrant treatment in the presence or absence of 10 nM E2. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **(E)** Cell motility was detected using scratch wound assays in hormone-deprived T47D cells stably expressing an *ESR1* construct and treated with 50 ng/ml mitomycin C to block proliferation (mean $\pm$ SEM, n=3). One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare each stable T47D cell line to YFP control cells (ns: not significant). Cells are pseudo-colored orange to aid visualization.

Supplementary Figure 6, related to Figure 5



Supplementary figure 6. Inactive ESR1-TCF12 fusion protein binds estrogen response element (ERE)-containing DNA like active *ESR1* fusion proteins. Nuclear extracts of T47D cell lines expressing YFP, ESR1-e6>YAP1, ESR1-e6>SOX9, ESR1-e6>CLINT1, or ESR1-e6>TCF12 were prepared as described in the Methods section. All *ESR1* fusion-expressing cells were treated with 100 nM fulvestrant (Fulv) overnight to reduce the endogenous ER α level and thus its competition for binding to the EREs. ERE DNA pulldowns were done as described in the Methods section. *Top panel*, Immunoblotting of ER α and *ESR1* fusion proteins with an N-terminal ER α antibody reveals levels in 2% of starting inputs (*left*) and what was bound to 30% of the final 4xERE DNA beads (*right*). Asterisks indicate ER fusion proteins. *Bottom panel*, Immunoblotting of DNA-PK catalytic subunit (DNA-PKcs) serves as a loading control. The dashed line indicates two separate blots that were conducted at the same time.

3) Supplementary Table

Supplementary table 1. Comprehensive summary of *ESR1* gene fusions in ER+ breast cancer

<i>ESR1</i> fusions	Frame	Examples	Source	Mechanisms
Active fusions	In-frame	ESR1-e6>YAP1 (4,5) ESR1-e6>PCDH11X (5) ESR1-e6>SOX9 (6) ESR1-e6>ARNT2-e18* ESR1-e6>ARNT2-e2 (7) ESR1-e6>LPP (7) ESR1-e6>NCOA1 (7) ESR1-e6>CLINT1 (7) ESR1-e6>GRIP1 (7) ESR1-e6>TNRC6B (7)	Metastatic tumors	Generated by inter-chromosomal translocation Produce stable ER α fusion protein Upregulate transcriptional activation of ER α target and EMT genes Drive ET-resistant tumor growth and metastasis
	5'UTR-CDS	ESR1-e2>CCDC170 (8) ESR1-e2>C6orf211 (9)	Primary tumors	Generated by tandem duplication Produce truncated partner protein (rather than a chimeric protein) Reduce ET sensitivity
Cell context-dependent fusion	In-frame	ESR1-e6>DAB2 (6)	Metastatic tumors	Generated by inter-chromosomal translocation Produce stable ER α fusion protein Drive hormone-independent growth in MCF7 but not T47D cells
Inactive fusions	In-frame	ESR1-e6>GYG1 (6) ESR1-e6>PCMT1* ESR1-e6>ARID1B* ESR1-e6>TCF12 (7)	Metastatic tumors	Generated by inter-, or intra-chromosomal translocation Produce stable ER α fusion protein Do not drive E2-independent growth
		ESR1-e6>NOP2 (5) ESR1-e7>POLH (5) ESR1-e6>AKAP12 (5,9)	Primary tumors	Generated by inter-chromosomal translocation or tandem duplication Do not drive E2-independent growth
	Out-of-frame	ESR1-e3>CCDC170 (5) ESR1-e4>CCDC170 (5) ESR1-e5>CCDC170 (5) ESR1-e6>AKR1D1 (5)		

*Personal communication, Dr. Dan Robinson

4) Supplementary References

1. Peters TL, Kumar V, Polikepahad S, Lin FY, Sarabia SF, Liang Y, *et al.* BCOR-CCNB3 fusions are frequent in undifferentiated sarcomas of male children. *Mod Pathol* **2015**;28:575-86
2. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **2010**;26:139-40
3. Rokita JL, Rathi KS, Cardenas MF, Upton KA, Jayaseelan J, Cross KL, *et al.* Genomic Profiling of Childhood Tumor Patient-Derived Xenograft Models to Enable Rational Clinical Trial Design. *Cell Rep* **2019**;29:1675-89 e9
4. Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, *et al.* Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep* **2013**;4:1116-30
5. Lei JT, Shao J, Zhang J, Iglesia M, Chan DW, Cao J, *et al.* Functional Annotation of ESR1 Gene Fusions in Estrogen Receptor-Positive Breast Cancer. *Cell Rep* **2018**;24:1434-44 e7
6. Hartmaier RJ, Trabucco SE, Priedigkeit N, Chung JH, Parachoniak CA, Vanden Borre P, *et al.* Recurrent hyperactive ESR1 fusion proteins in endocrine therapy-resistant breast cancer. *Ann Oncol* **2018**;29:872-80
7. Priestley P, Baber J, Lolkema MP, Steeghs N, de Bruijn E, Shale C, *et al.* Pan-cancer whole-genome analyses of metastatic solid tumours. *Nature* **2019**;575:210-6
8. Veeraraghavan J, Tan Y, Cao XX, Kim JA, Wang X, Chamness GC, *et al.* Recurrent ESR1-CCDC170 rearrangements in an aggressive subset of oestrogen receptor-positive breast cancers. *Nat Commun* **2014**;5:4577
9. Giltmane JM, Hutchinson KE, Stricker TP, Formisano L, Young CD, Estrada MV, *et al.* Genomic profiling of ER(+) breast cancers after short-term estrogen suppression reveals alterations associated with endocrine resistance. *Sci Transl Med* **2017**;9

FOULDS, CHARLES E.

Active

Foulds College Seed
Baylor College of Medicine

07/01/2021 - ongoing

0.00 CM

03/15/21 – 03/14/24

W81XWH-21-1-0119 (Foulds) **This Award**

4.80 CM

Department of Defense

Project Title: Proteogenomic approaches for finding therapeutic vulnerabilities to treat breast tumors expressing transcriptionally active ESR1 fusions.

Contact: Jamie A. Shortall – Jamie.a.shortall.civ@mail.mil

Role: Co-Investigator

Major goals: Our proposed research will promote the ability to diagnose active *ESR1* fusions accurately in the clinic (by developing an “active *ESR1* gene fusion signature”) and will reveal specific kinase and coactivator inhibitor- based therapeutic vulnerabilities for the treatment of *ESR1* fusion-driven metastatic breast cancer. Our pre-clinical therapeutic data will support the development of future clinical trials for patients expressing active *ESR1* fusions that cannot be treated by existing endocrine therapies.

Specific Aims:

Aim 1: To establish functional rules that predict which ESR1 fusions are active drivers of drug resistance and metastasis

Aim 2: To investigate ESR1 fusion-activated downstream kinases for therapeutic targeting

Aim 3: To identify common coactivators recruited by ESR1 fusions as therapeutic targets

Adrienne Helis Malvin Medical Research Foundation (Foulds) **Awarded** 10/01/20 – 09/30/23
3.60 CM

Project Title: Kinase inhibition for ESR1 fusion-driven breast cancer

Contact: Kimberlee Townsend ktownsend@helisoil.com

Role: Co-Investigator

Major Goals: This proposal provides strong evidence that *ESR1* translocation events are an emerging class of recurrent somatic mutations that lead to not only therapeutic drug resistance but also lethal metastasis in a subset of patients with ER+ breast cancer, a disease not previously considered to be driven by gene fusions. *These ESR1 fusions cannot be treated with the current standard-of-care ET, as they produce proteins lacking the ERα LBD.* However, we will define gene expression patterns that classify pathogenic *ESR1* fusion proteins and their downstream activated kinases to allow new therapeutics to be developed to treat *ESR1* translocated breast tumors. Results of this study will also shed new mechanistic insight into how ER+ breast cancer becomes ET-resistant and metastatic, a lethal process that is still poorly understood.

Development Research Projects (DRP) **New**

07/01/21 – 06/30/22

0.36 CM NIH/

NCI

Project Title: Translational Research in Breast Cancer

Contact: Funmi Elesinmogun; Email: elesinmf@mail.nih.gov

Role: PI

Major Goals: For the fastest and most efficient progress against breast cancer, we need to draw the broadest possible range of ideas and approaches into the fight. SPORE developmental projects allow us to take on high risk research and attract new ideas, so that the most promising can earn support to become larger successful efforts either within the SPORE or with other major funding.

Pending

None

Completed in Last 5 Years

RP180844 (Eric Chang, BCM)

08/31/2018 - 08/30/2021

0.36 CM

Cancer Prevention and Research Institute of Texas (CPRIT)

Project Title: Regulating Androgen Receptor as a Corepressor by Neurofibromin (NF1)

Contact: Patty Moore; Email: pmoore@cprit.texas.gov

Role: Co-Investigator

Major Goals: The major goal is to investigate whether NF1 directly regulates androgen receptor (AR) in breast cancer cells, and whether anti-AR agents could therefore have a significant role in suppressing aggressive growth of NF1-deficient breast cancer.

Specific Aims

1. We will investigate whether NF1 directly regulates AR in breast cancer cells
2. We will investigate whether anti-AR agents have a significant role in suppressing aggressive growth of NF1-deficient breast cancer.

Cancer Program M-2017-2 (Foulds) **Ended**

04/01/17-03/31/21 NCE

0.00 CM

Adrienne Helis Malvin Medical Research Foundation

Project Title: Coactivator-Based Therapy for ER Mutant Breast Cancers

Contact: David A. Kerstein; Email: helisenergyproject@gmail.com

Role: PI

Major Goals: The main goals of this project is to identify coactivator proteins interacting with estrogen receptor- α (ER α) ligand binding domain (LBD) mutant proteins and to then test if steroid receptor coactivator inhibitors will reduce tumor growth driven by ER α LBD mutants.

Specific Aims

1. To define the complement of coactivator protein-protein interactions with WT and mutant ER α bound to EREs
2. To determine whether a novel strategy that targets SRC coactivators will reduce the growth of breast cancers expressing mutant ER α proteins.

Gulf Coast Center for Precision Environmental Health NIEHS Center Pilot Project (part of P30ES030285)

11/01/19–10/31/20 (Foulds, Hodges) **Ended**

Project Title: Defining how an environmental “obesogen” impacts the transcriptional and chromatin landscape of human liver cells to promote steatosis

Contact: Heyreoun An Han, Ph.D.; Email: Heyreoun.Han@bcm.edu

Role: PI

Major Goals: The major goal is to test whether tributyltin (TBT) may induce a pro-lipogenic transcriptional program in human liver cells (HepaRG and primary hepatocytes) by promoting an open chromatin state that is modulated by ERK kinase signaling.

Specific Aims

1. Identify coregulators recruited to the *ANGPTL4* enhancer in a TBT and ERK kinase-dependent manner.
2. Elucidate how TBT affects gene expression and the genome-wide chromatin landscape in an ERK-dependent manner.

RP 150648-P2 (Nancy Weigel, BCM)

06/01/2015- 05/31/2020

0.6 CM

Cancer Prevention Research Institute of Texas (CPRIT)

Project Title: Project 2: Characterization of the AR-V7 Cistrome, Transcriptome and Proteome

Contact: Michael Brown; Email: mbrown@cprit.state.tx.us, 512-305-7676

Role: Co-Investigator

Major Goals: The goal of this project in the larger CPRIT MIRA (O'Malley, PI, "GATA2 and Steroid Receptor Coactivator-2 Cooperate With Androgen Receptor in Prostate Cancer Progression and Androgen Resistance") is characterization of the androgen receptor variant V-7 cistrome, transcriptome and proteome.

Specific Aim

1. We will define where AR V-7 binds in the genome, what genes it regulates, and what coregulators it binds in prostate cancer cells.

W81XWH-16-1-0538 (Eric Chang, BCM)

09/30/2016 – 10/01/2019

0.6 CM

Department of Defense, DOD No. BC151851

Project Title: Direct Regulation of Estrogen Receptor Transcriptional Activity by NF1

Contact: Jamie A. Shortall – Jamie.a.shortall.civ@mail.mil

Role: Co-Investigator

Major Goals: The overall research goal is to discover drivers for endocrine therapy in ER+ breast cancer, which account for the majority of deaths from the disease. We have evidence that loss of NF1 promotes endocrine therapy resistance and leads to relapse and death.

Specific Aims

1. We will define how NF1 regulates expression of ER target genes by investigating a direct interaction between NF1 and canonical ER transcriptional co-regulators.
2. We will establish a strategy to effectively treat NF1-deficient ER+ breast cancers by rationally combining anti-Ras and anti-ER approaches. We will first determine what drug combination works best in vitro and will then further investigate this in vivo using cell line and patient-derived xenograft mouse models.

1R01DK108346 (Michael J. Ross, Albert Einstein College of Medicine) 07/1/2015 – 06/30/2019

NIH/NIDDK (Subcontract to BCM)

1.2CM

Project Title: The role of APOL1 polymorphisms in promoting HIV-related kidney injury

Contact: Grants Management Specialist: Charlette Kenley; Email: kenleyc@extra.niddk.nih.gov; Phone: (301) 594-8847; Fax: (301) 480-3504

Role: PI of subcontract

Major Goals: The goal is determine what proteins interact with wild-type APOL1 and two risk alleles (G0, G1) during HIV-infection, as the risk alleles have worse prognosis for kidney injury and the mechanism for this promotion remains unresolved.

Specific Aims

1. We will define the protein "complexome" for wild-type APOL1 and two risk allele APOL1 variants by immunoprecipitation- mass spectrometry.

W81XWH-16-1-0297 (Subhamoy Dasgupta, BCM)

07/01/2016- 06/30/2019

0.6 CM

Department of Defense, DOD No. PC150115

Project Title: Targeting Metabolic Adaptation Pathways to Inhibit Progression and Metastasis of Castration-Resistant Prostate Cancer

Contact: Grants Management Specialist: Asif Rizwan, PhD, Science Officer, Phone: (301) 619-4398, E-mail: asif.m.rizwan.ctr@mail.mil

Role: Co-Investigator

Major Goals: The major goal of this grant is to test the hypothesis that increased glutamine metabolism is an oncogenic driver of CRPC-emergence, nominating it as an attractive therapeutic target to inhibit prostate cancer metastasis. Our objectives in this proposal are to decipher the molecular links between cellular metabolism and oncogenic events associated with the progression of an organ confined prostate cancer into a metastatic disease, and exploit the atypical glutamine metabolism in mCRPC as a means of therapeutic intervention by using a glutaminase inhibitor.

Specific Aims

1. To define the mechanisms of coactivator-SRC-2 dependent metabolic reprogramming in castration-resistant

prostate tumors.

2. To investigate the importance of glutamine metabolism as a critical determinant of CRPC emergence.
3. To determine whether inhibition of glutamine catabolism can selectively impair prostate tumor progression and metastasis in vivo.

NURSA Consensome Validation NDSPs (NIH/NIDDK) 9/01/2017 – 8/31/2018

1.2CM

4 U24 DK097748-05 (Subaward to C. Foulds)

Project Title: Role of Novel PPAR-RXR Target Genes in Liver Reprogramming by Obesogens

Contact: Corinne M. Silva, Ph.D., Program Director, NURSA 3.0, DEM/NIDDK/NIH, Ph: 301-451-7335, Fax: 301-480-0475, Email: silvacm@mail.nih.gov

Role: PI

Major Goals: The major goal is to validate whether three obesogens (TBT, PFOA, and PFOS) similarly regulate 9 suggested PPAR pathway genes from the Consensome website (and two known PPAR targets) in HepaRG cells and will test the effect of loss of PPARs and RXRs on their expression patterns. Furthermore, the effect of knockdown of these downstream genes on lipid accumulation will be assayed. Finally, whether obesogen binding changes the chromatin occupancy of PPARs and RXR at these target genes will be determined.

Specific Aims

1. Determine if obesogens regulate 9 predicted TBT-induced genes in a similar fashion in a PPAR-RXR dependent manner and how loss of expression of these genes impacts lipid accumulation.
2. Identify if obesogens affect the predicted TBT-induced PPAR-RXR genes differently than established ligands for PPAR-RXR heterodimers and if they change the chromatin occupancy of PPAR-RXR at these genes.

DOD No. BC120894 (Bert W. O'Malley, BCM)

09/01/2013 – 08/31/2018

4.8CM

Department of Defense

Project Title: Development of Coactivator-Dependent, First-in-Class Therapies for Breast Cancer

Contact: JODI.LCARDOZA.CIV@MAIL.MIL, (301) 619-2693

Role: Co-Investigator

Major Goals: The major goal of this grant is to identify accurate predictors and novel therapeutic drug approaches to prevent and overcome the development of late recurrence and hormone-resistant metastases of breast cancers.

Specific Aims

1. We plan to develop SRC-3 SMIs that will go on to find a place as first-in-class chemotherapeutic agents that, in combination with existing endocrine, targeted and chemotherapeutic agents, can be used to treat breast cancer patients.

1R01ES023206-01 (Cheryl Walker, BCM)

09/01/2013 – 05/31/2017

1.2CM

NIH, National Institute of Environmental Health Sciences (NIEHS)

Project Title: Epigenetic Programmers Targeted During Developmental Reprogramming

Contact: Grants Management Specialist: Michelle Victalino, Email: victalinom@od.nih.gov

Role: Co-Investigator

Major Goals: The goal is determine how four endocrine disrupting chemicals (EDCs), relevant to human exposure, disrupt the normal function of epigenomic programmers in the liver and how this perturbation reprograms hepatic metabolic programs resulting in obesity.

Specific Aims

1. Identify pre-genomic signaling pathways activated by obesogenic EDCs in the liver that target epigenomic “readers, writers, and erasers” and determine how induction of post-translational modifications (PTMs) disrupt the activity of these epigenomic programmers.
2. Test the hypothesis that obesogenic EDCs alter nuclear hormone receptor (NHR)-coregulator complex formation and/or PTMs of coregulators to disrupt “classical” NHR genomic signaling.
3. Test the hypothesis that in the neonatal liver, EDC-directed PTM of epigenetic programmers alters their activity to reprogram the liver toward a metabolic set-point favoring obesity.

Center for Translational Environmental Health Research (CTEHR) (Foulds) 01/01/2016-3/31/2016

Pilot Project Program Grant Subaward No.: 23-S162332

(no salary support)

(Supported by NIEHS P30ES023512)

Project Title: Characterization of the endocrine disruptor tributyltin on nuclear receptor-coregulator complex formation and the promotion of adipogenesis

Contact: Dan Na Luo; Center for Translational Environmental Health Research; Email: dluo@ibt.tamhsc.edu; Phone: 713-677-7757

Role: PI

Major Goals: The goal of this PPP is to define the suite of coactivators recruited to two NRs (PPAR γ /RXR α) in adipocytes exposed to a model obesogenic EDC, tributyltin (TBT), relevant to human exposure.

Specific Aims

1. I will define the coregulator “complexome” induced by TBT binding PPAR/RXR on DNA using mass spectrometry.

Overlap

None

ANURAG, MEENAKSHI

ACTIVE

U01 CA214125 (Anurag/Carr) **Now PI**
NCI

06/01/2017 – 05/31/2022

2.16 CM NIH/

Project Title: (MPI) Microscaled Proteogenomics for Cancer Clinical Trials (CPTAC)

Contact: Viviana Knowles; Email: Viviana.Knowles@nih.gov

Role: Bioinformatician

Major Goals: The overall goal of our proposal leverages state-of-the-art quantitative discovery proteomics and phosphoproteomics as well as targeted assays to measure the kinome and chromatin modifications. These sensitive and reproducible pipelines will be used to analyze preclinical models, well-annotated cohorts and clinical trial samples in an iterative design. A robust proteogenomics pipeline developed by our group will be used to analyze and visualize the data.

Specific Aims:

1. Determine the adequacy of our microscaled proteomic pipelines and PDX resources in revealing tumor biology.
2. Develop a prioritization scheme for clinical trial sample analysis using Tier 2 (core needle biopsies from small-scale neoadjuvant studies samples)
3. Determine interactions between the proteogenome, drug response and outcomes in breast cancer neoadjuvant clinical trials.

Overlap: None

U54CA233223 (Mitsiades) **Reduced effort**
NCI

09/20/2018 – 06/30/2023

2.16 CM NIH/

Project Title: Minority PDX Development and Trial Center: Baylor College of Medicine and MD Anderson Cancer Center Collaboration in Mechanistic Studies to Dissect and Combat Health Disparities in Cancer RP2: Targeting Estrogen receptor and DNA damage repair disparities in African American and Hispanic/Latino breast cancer using Patient-Derived Breast Cancer Xenografts

Contact: Ashley Salo - ashley.salo@nih.gov

Role: Bioinformatician

Major Goals: The objective of this study is to characterize the genome, transcriptome and kinome of 100 breast cancer PDX lines, >70% of which are derived from patients of African American or Hispanic/Latino ethnicity. By understanding fundamental cancer pathways specific to these minority groups this study will delineate efficacy of pre-existing, CTEP-approved therapies in minority ethnicities.

Specific Aims:

1. Systematically characterize estrogen receptor (ER) signaling and response to endocrine treatment in PDX lines derived from AA and Hispanic breast cancer patients.
2. Characterize DNA repair profiles of ER+ and triple negative PDX lines across ethnic groups.
3. Generate -omics' based network profiles specific for Hispanic, AA and CA PDXs.

Overlap: None

SAC190059 – Leadership Grant (Ellis) **Reduced effort**
Susan G Komen Foundation

06/01/19 – 12/31/2021

0.12 CM

Project Title: Proteogenomics of Endocrine Therapy Resistance

Contact: Amy Dworkin; Email: Adworkin@komen.org

Role: Co-Investigator

Specific Aims:

1. Conduct Tandem-Mass Tag (TMT) quantitative proteomic and phosphoproteomic analysis of ER+ PDX grown in the presence and absence of estradiol supplementation. Informatic tools will be applied to contrast estradiol-dependent and independent models and metastatic and non-metastatic models

2. Tractable therapeutic hypotheses that are specific to pathways that are active in the hormone independent state will be identified. We will conduct proof of principle therapeutic experiments in endocrine therapy resistant PDX models to achieve validation of the predictive properties of proteogenomic analyses conducted in Aim 1.

Overlap: None

Adrienne Helis Malvin Medical Research Foundation (Foulds) **New** 10/01/20 – 09/30/23 0.12 CM

Project Title: Kinase inhibition for ESR1 fusion-driven breast cancer

Contact: Kimberlee Townsend ktownsend@helisoil.com

Role: Co-Investigator

Major Goals: This proposal provides strong evidence that *ESR1* translocation events are an emerging class of recurrent somatic mutations that lead to not only therapeutic drug resistance but also lethal metastasis in a subset of patients with ER+ breast cancer, a disease not previously considered to be driven by gene fusions. *These ESR1 fusions cannot be treated with the current standard-of-care ET, as they produce proteins lacking the ERα LBD.* However, we will define gene expression patterns that classify pathogenic *ESR1* fusion proteins and their downstream activated kinases to allow new therapeutics to be developed to treat *ESR1* translocated breast tumors. Results of this study will also shed new mechanistic insight into how ER+ breast cancer becomes ET-resistant and metastatic, a lethal process that is still poorly understood.

P50 CA186784-06 (Ellis)

08/01/20 – 07/31/25

1.20 CM NIH/

NCI

Project Title: Translational Research in Breast Cancer

Contact: Funmi Elesinmogun; Email: elesinmf@mail.nih.gov

Role: Co-Director – Biostatistics, Information, and Computational Biology (Core B)

This Core provides comprehensive and essential statistical, bioinformatics, medical informatic and data management support to all projects, to the DRP and CEP and to the other Cores. Our mission is to bring the best possible methods to bear, and to help ensure that the translational goals of the SPORE will be met while making efficient use of resources.

Specific Aims:

1. Comprehensive biostatistical consultation, experimental design, data analysis and reporting;
2. Integrative proteome-genomic bioinformatic consultation, experimental design, data analysis and reporting;
3. Development, customization, integration and maintenance of databases and data management systems to support data management needs of SPORE projects and cores.

Overlap: None

BCC Philanthropic Project (Learner) **New**

08/01/20 – 7/31/25

0.96 CM BCM

Partnership for Bladder Cancer Research

Project Title: Translational Bladder Cancer Research

Contact: Karoline Kremers; Email: Karoline.Kremers@bcm.edu

Role: Bioinformatics

Vision for the Bladder Cancer Program at Baylor College of Medicine is to accelerate innovation in research and treatment and invent the future of personalized precision care for bladder cancer patients

Specific Aims:

1. Comprehensive characterization 350 NMIBC including 100 low risk (Ta low grade), 100 intermediate risk (multifocal and/or recurrent Ta low grade), and 150 high risk (Ta or T1HG, Tis) divided equally between treatment naïve and previously treated.
2. Comprehensive characterization of carcinoma in situ: up to 100 samples stratified between treatment naïve and recurrence post BCG.
3. Functional validation a) miRNA project; b) pre-clinical models – PDX; organoids; c) Phase O platform for evaluating novel agents and biomarkers

4. Comprehensive integrated bioinformatics and biobanking

W81XWH-21-1-0634 (Chang) New

08/01/21 – 07/31/23

1.20 CM

Department of Defense

Project Title: Optimizing Treatment for NF1-Deficient Metastatic ER+ Breast Cancers (Expansion Award)

Contact: Amanda Carrera - amanda.c.carrera.civ@mail.mil

Role: Co-Investigator - W81XWH-20-BCRP-EA

Major Goals: The major goal is to further interrogate approaches to diagnose NF1 loss in clinical samples in order to expand eligibility criteria for trials targeting ER+ breast cancer with NF1 loss.

Specific Aims:

Aim 1: we will further assess whether this MS-based diagnostic approach can adequately assess NF1 protein levels in patients who will be selected first by DNA sequencing.

Aim 2: we will functionally characterize these NF1 mutants to assess whether they should be included in future trials because, despite being mis-sense they disrupt NF1 function in a manner that sensitizes to the fulvestrant/binimetinib combination.

W81XWH-21-1-0119 (Foulds) This Award

03/15/21 – 03/14/24

1.80 CM

Department of Defense

Project Title: Proteogenomic approaches for finding therapeutic vulnerabilities to treat breast tumors expressing transcriptionally active ESR1 fusions.

Contact: Jamie A. Shortall – Jamie.a.shortall.civ@mail.mil

Role: PI

Major goals: Our proposed research will promote the ability to diagnose active *ESR1* fusions accurately in the clinic (by developing an “active *ESR1* gene fusion signature”) and will reveal specific kinase and coactivator inhibitor- based therapeutic vulnerabilities for the treatment of *ESR1* fusion-driven metastatic breast cancer. Our pre-clinical therapeutic data will support the development of future clinical trials for patients expressing active *ESR1* fusions that cannot be treated by existing endocrine therapies.

Specific Aims:

Aim 1: To establish functional rules that predict which ESR1 fusions are active drivers of drug resistance and metastasis

Aim 2: To investigate ESR1 fusion-activated downstream kinases for therapeutic targeting

Aim 3: To identify common coactivators recruited by ESR1 fusions as therapeutic targets

W81XWH-21-1-0107 (Lim) New

03/01/21 – 12/31/24

0.60

CM

Department of Defense

Project Title: Therapeutic Targeting of Nuclear Hormone Receptors in Neurofibromin/NF1-Depleted Breast Cancer

Contact: Amanda Carrera - amanda.c.carrera.civ@mail.mil

Role: Co-Investigator

Major Goals: The overall objective of this project is to investigate this hypothesis in order to assess what therapeutic opportunities are associated with neurofibromin loss in patients

Specific Aims:

Aim 1: we will define how neurofibromin and AR interact by investigating the role of neurofibromin as an AR co-repressor. We will measure direct ligand-dependent interaction between neurofibromin and AR on the DNA, and the consequence on gene expression when neurofibromin is lost in AR+ ER+, as well as ER–, breast cancer cells

Aim 2: we will study how AR antagonists (e.g., enzalutamide) or less virilizing Selective Androgen Receptor Modulators (e.g., ostarine/enobosarm) can impact the growth of breast cancer cells upon neurofibromin-depletion using cell line and PDX models.

P50 CA186784 (Ellis) New

07/01/21 – 06/30/22

0.48 CM NIH/

NCI

Project Title: Translational Research in Breast Cancer – Career Enhancement Program (CEP)

Contact: Funmi Elesinmogun; Email: elesinmf@mail.nih.gov

Role: PI

Major Goals: Career Enhancement Program (CEP). The central goal of our translational research is to get the most up to date laboratory research and the most current clinical experience to talk productively to each other, for the most rapid and efficient progress toward controlling and eliminating breast cancer. Thus we have designed this career enhancement program to expose young researchers to the full range of the breast cancer research experience in a vigorously translational environment, whatever their initial training was, as they move towards research independence

R01 CA271498-01 (Li/Zhang) New

03/14/2022 – 03/13/2027

1.20 CM NIH

Project Title: Next Generation Rat Models of ER+ Breast Cancer

Contact: Shakeeya Mone Eaddy; Email: shakeeya.eaddy@nih.gov

Role: Co-Investigator

Major Goals: The major goal of this proposal is to develop and credential rat models of ER+ breast cancer for studying ER+ breast cancer progression, metastasis and therapeutic resistance.

Specific Aims: (1) To characterize early progression of ER+ BCa in RIIM models.

(2) To characterize the metastatic behaviors of ER+ BCa in RIIM models. (3) To credential ER+ RIIM models in recapitulating therapeutic responses

Overlap: None

PENDING

None

Overlap:

None

COMPLETED

W81XWH-19-1-0527 – BC181527 (Chang)

09/01/19 – 08/31/2021

Department of Defense

Project Title: Direct Regulation of Estrogen Receptor Transcription Activity by NF1 (Expansion Award)

Contact: Jodi Cardoza; Email: Jodi.l.cardoza.civ@mail.mil

Role: Bioinformatician

Specific Aims:

AIM 1: To define the full range of NF1 transcriptional activity in ER+ breast cancer cells by identifying key metastasis-driving genes that are directly regulated by NF1.

AIM 2: To assess the impact of NF1 depletion on bone metastasis using BICA (bone in culture assay) in vitro and IIA (intra iliac artery) injection in vivo, and how to block these activities in order to reduce metastasis in ER+ NF1–cancer.

Role: Bioinformatician

SAC170059 – Leadership Grant (Ellis)

02/20/2017 – 02/19/2019

3.0 CM Susan

G. Komen

Project Title: Somatic Mutation and Recurrence Risk for Early Stage Estrogen Receptor Positive Breast Cancer

Contact: Jerome Jourquin; Email: JJourquin@komen.org

Role: Biostatistician

Major Goals: The primary objective of this proposal is to establish relationships between somatic mutations in significantly mutated or druggable genes in breast cancer and outcomes for patients receiving adjuvant endocrine therapy.

Specific Aims:

1. We will establish the cell line models to define the molecular activities caused by NF1 inactivation.
2. We will use cell lines identified in Aim 1, as well as ER+ PDXs (patient-derived xenografts) that have NF1 mutations, to investigate whether greater efficacy in standard endocrine therapy can be achieved by also targeting the NF1-dependent Ras pathways.

Overlap: None

RR140033 (Ellis)

06/01/2014 – 11/30/2019 NCE

2.68 CM

CPRIT

Established Investigator Recruitment Award

Project Title: Proteogenomic and genomic analysis of luminal and triple negative breast cancer for targeted therapeutics discovery.

Contact: Michael Brown; Email: mbrown@cprit.state.tx.us

Role: Bioinformatician

Specific Aims:

1. Develop a mechanism-based classification of endocrine therapy resistant ER+ HER2- breast cancer and translate these findings into improved clinical outcomes through clinical trials in the neoadjuvant and metastatic settings
2. Develop a mechanism-based classification of chemotherapy resistant ER- HER2- breast cancer and translate these findings into improved clinical outcomes through clinical trials in the neoadjuvant and metastatic settings
3. Serve goals 1 and 2 through the development of clinically actionable CLIA and/or FDA approved tests based on the measurement of critical DNA, RNA, protein and post-translational modifications that define druggable biology

Overlap: None

W81XWH-19-1-0527 – BC181527 (Chang)

09/01/19 – 08/31/2021

0.6 CM

Department of Defense

Project Title: Direct Regulation of Estrogen Receptor Transcription Activity by NF1 (Expansion Award)

Contact: Jodi Cardoza; Email: Jodi.l.cardoza.civ@mail.mil

Role: Bioinformatician

Specific Aims:

AIM 1: To define the full range of NF1 transcriptional activity in ER+ breast cancer cells by identifying key metastasis-driving genes that are directly regulated by NF1.

AIM 2: To assess the impact of NF1 depletion on bone metastasis using BICA (bone in culture assay) in vitro and IIA (intra iliac artery) injection in vivo, and how to block these activities in order to reduce metastasis in ER+ NF1– cancer.

Role: Bioinformatician

SUSAN HILSENBECK

ACTIVE

U54CA233223 (Mitsiades)

09/20/18 – 06/30/23

1.20 CM

NIH

Project Title: Minority PDX Development and Trial Center: Baylor College of Medicine and MD Anderson Cancer Center Collaboration in Mechanistic Studies to Dissect and Combat Health Disparities in Cancer RP2: Targeting Estrogen receptor and DNA damage repair disparities in African American and Hispanic/Latino breast cancer using Patient-Derived Breast Cancer Xenografts

Contact: Ashley Salo - ashley.salo@nih.gov

Role: Co-Investigator

Major Goals: The objective of this study is to characterize the genome, transcriptome and kinome of 100 breast cancer PDX lines, >70% of which are derived from patients of African American or Hispanic/Latino ethnicity. By understanding fundamental cancer pathways specific to these minority groups this study will delineate efficacy of pre-existing, CTEP--approved therapies in minority ethnicities.

Specific Aims:

1. Systematically characterize estrogen receptor (ER) signaling and response to endocrine treatment in PDX lines derived from AA and Hispanic breast cancer patients.
2. Characterize DNA repair profiles of ER+ and triple negative PDX lines across ethnic groups.
3. Generate -omics' based network profiles specific for Hispanic, AA and CA PDXs.

Overlap: None

R01AR072018 (Park, D)

07/09/18 - 06/30/23

0.60 CM NIH

Project Title: Defining periosteal skeletal stem cells and novel migration mechanisms in bone regeneration and repair in vivo

Contact: Fei Wang; Email: wangf@mail.nih.gov

Role: Co-Investigator

The major goal of this project is to define in vivo function of periosteal SSCs and the mechanisms that regulate P-SSCs in bone regeneration and repair. These studies will further define new therapeutic targets for reversing bone diseases and defects.

Specific Aims:

1. Determine whether inflammatory stimuli such as CCL5 are necessary for P-SSC migration and bone healing in vivo.
2. Test whether local ablation of P-SSCs or CCR5 deletion in P-SSCs affect bone healing in vivo.
3. Determine whether local provision of CCL5 accelerates healing of aged-bone defect with increased P-SSC recruitment but decreased osteoclast activity.

Overlap: None

W81XWH-17-1-0579 (Rimawi)

09/15/17 - 09/14/22

0.60 CM

DOD

Project Title: A New Paradigm for De-escalation of Treatment in Her2 Positive Breast Cancer: Revolutionizing Care with More Effective and Less Toxic Therapy

Contact: Elayne Seiler; Email: Elayne.K.Seiler.civ@mail.com; Phone: 301-619-7358

Role: Co-Investigator

Major Goals: The overall objective of this proposal is to define, and functionally characterize, mechanisms of resistance to dual anti-HER2 therapy, and use this information to develop a multi-parameter classifier to assign patients into distinct therapeutic groups on a prospective clinical trial.

Specific Aims:

1. To identify and validate additional genes/pathways and genomic alterations associated with resistance (LTR)

2. To conduct a clinical trial that will test the feasibility and provide early results of a next generation clinical trial design that uses tumor characteristics to successfully perform a molecular triage that identifies patients who can benefit from anti-HER2 therapy alone without chemotherapy

Overlap: None

U54CA224076 (Welm/Lewis)

09/01/17 - 08/31/22

0.33 CM NIH

Project Title: (MPI) PDX Trial Center for Breast Cancer Therapy

Contact: Jacquelyn Saval Email: boudjedaj@mail.nih.gov Phone: (240)276-6312

Role: Co-Investigator

Major Goals: The proposed Patient-derived Xenograft Development and Trials Center (PDTC) will be a multi-institutional Center comprising three well-established research groups: two from the Huntsman Cancer Institute (HCI), and one from Baylor College of Medicine (BCM). The scientific premise of this study is that breast PDX models represent the diversity of human breast tumors, and can be feasibly utilized to test new drugs and drug combinations to find therapeutic approaches that match with molecular features of various tumor types. The goal of our PDTC is to obtain preclinical data that will facilitate prioritization of drugs to be tested in clinical trials at the National Cancer Institute (NCI). Toward this goal, our PDTC focuses on testing drugs that are already available through the NCI as NCI-IND agents with the Experimental Therapeutics Clinical Trials Network (ETCTN).

Overlap: None

R01MD013715 (Montealegre)

04/16/19 – 12/31/23

0.60 CM NIH

Project Title: A Randomized Controlled Trial of Mail-Self Stamped HPV Testing to Increase Cervical Cancer Screening Participation Among Minority/Underserved Women in an Integrated Safety Net Healthcare System

Contact: Carla Griffith; Email: griffithcp@mail.nih.gov; Phone: 301-594-8944

Role: Co-Investigator

The major goal of the project is to create a process for cost-effective strategies to improve existing screening programs that involve testing self-collected cervicovaginal samples for HR-HPV to overcome the multiple barriers to clinic-based screening.

Specific Aims:

1. Compare the effectiveness of mailed self-sample HPV testing alone and in combination with patient navigation to increase primary screening participation (primary outcome) and clinical follow-up (secondary outcome).
2. Describe attitudes and experiences toward screening among women who receive mailed self-sampling kits and toward clinical follow-up among those who test positive for HR-HPV.
3. Evaluate the cost-effectiveness of mailed self-sample HPV testing alone and in combination with patient navigation to increase screening participation and reduce cervical cancer risk in safety net health systems.

Overlap: None

RP190398 (Schiff) **Now at NCE**
CPRIT

03/01/19 – 02/28/23 NCE

0.12 CM

Project Title: Targeting the Mechanism of Hyperactive FOXA1 in Transcriptional Reprogramming Toward Endocrine Resistance and Metastasis in Breast Cancer

Grant Contact: Jim Willson; jwillson@cpritis.texas.gov

Role: Co-Investigator

Specific Aims:

1. Investigate and establish the role of H-FOXA1/AP-1 and ER and identify the key AP-1 components involved in promoting EndoR and metastatic propensities, using our diverse preclinical EndoR models.
2. Determine the mechanism by which H-FOXA1/AP-1 and ER form a core-regulatory circuit to amplify

transcriptional programs in EndoR and metastatic breast cancer.

3. Explore the therapeutic potential of targeting FOXA1/FRA1, and evaluate clinical importance of FOXA1/AP-1 and ER-dependent signatures in EndoR and metastatic ER+ breast cancer.

Overlap: None

U01 CA214125 (Anurag/Carr)

06/01/17 – 05/31/22

0.48 CM

NIH/NCI

Project Title: (MPI) Microscaled Proteogenomics for Cancer Clinical Trials (CPTAC)

Contact: Viviana Knowles; Email: Viviana.Knowles@nih.gov

Role: Co-Investigator

Major Goals: The overall goal of our proposal leverages state-of-the-art quantitative discovery proteomics and phosphoproteomics as well as targeted assays to measure the kinome and chromatin modifications. These sensitive and reproducible pipelines will be used to analyze preclinical models, well-annotated cohorts and clinical trial samples in an iterative design. A robust proteogenomics pipeline developed by our group will be used to analyze and visualize the data.

Specific Aims:

1. Determine the adequacy of our microscaled proteomic pipelines and PDX resources in revealing tumor biology.
2. Develop a prioritization scheme for clinical trial sample analysis using Tier 2 (core needle biopsies from small-scale neoadjuvant studies samples)
3. Determine interactions between the proteogenome, drug response and outcomes in breast cancer neoadjuvant clinical trials.

Overlap: None

Scalp Cooling Alopecia Prevention Trial H-33692

11/13/13 - 12/31/22

0.12 CM

Paxman Coolers Limited

Project Title: Scalp cooling for alopecia prevention (scalp)

Contact: Richard Paxman; Email: RichardPaxman@paxman-coolers.co.uk; Phone: 44-1484-349444

Role: Co-Investigator

Major Goal: The primary goal of this project is to demonstrate that the Orbis Paxman Hair Loss Prevention System is safe and effective in reducing chemotherapy-induced alopecia in woman with breast cancer undergoing neoadjuvant or adjuvant chemotherapy.

Specific Aim: Determine device is safe and effective at reducing chemotherapy-induced alopecia in women with breast cancer undergoing neoadjuvant or adjuvant chemotherapy

Overlap: None

P50CA126752 (Heslop/Brenner)

09/01/17 – 08/31/22

0.12 CM NIH-

NCI

Project Title: SPORE in Lymphoma – Competing Renewal

Contact: Viviana Knowles; Email: Viviana.knowles@nih.gov

Role: Core D – Co-Investigator

The overarching goal of this SPORE renewal proposal is to devise and test novel forms of cellular immunotherapy mediated by T cells and NKT cells to treat non-Hodgkin lymphoma (NHL) or Hodgkin lymphoma (HL).

Specific Aims:

1. To use highly specific T and NKT cellular immunotherapies to target multiple lymphoma antigens.
2. To increase the potency of the T and NKT cell immunotherapies for lymphoma.
3. Overcome the immune evasion tactics of lymphoma cells and their microenvironment.
4. To make T and NKT cell immunotherapy for lymphoma more broadly available.

Overlap: None

P50 CA186784 (Ellis)

08/01/20 – 07/31/25

1.20 CM

NIH/NCI

Project Title: Translational Research in Breast Cancer

Contact: Funmi Elesinmogun; Email: elesinmf@mail.nih.gov

Role: Core Director - Informatics and Statistics Core (Core B)

This Core provides comprehensive and essential statistical, bioinformatics, medical informatic and data management support to all projects, to the DRP and CEP and to the other Cores. Our mission is to bring the best possible methods to bear, and to help ensure that the translational goals of the SPORE will be met while making efficient use of resources.

Specific Aims:

1. Comprehensive biostatistical consultation, experimental design, data analysis and reporting;
2. Integrative proteome-genomic bioinformatic consultation, experimental design, data analysis and reporting;
3. Development, customization, integration and maintenance of databases and data management systems to support data management needs of SPORE projects and cores.

Overlap: None

U54CA254569 (Allen/Scheurer)

07/25/20 – 06/30/25

0.90 CM NIH

Project Title: Pediatric HIV/AIDS & Infection-Related Malignancies Research Consortium for Sub-Saharan Africa (PARCA)

Contact: Funmi Elesinmogun; Email: domingug@mail.nih.gov

Role: Co-Leader

Major Goals: The goal of this proposal is to establish PARCA, a collaborative clinical and translational research framework designed with the overarching goal to improve the current unacceptably poor outcomes of children with HIV-associated malignancies in sub-Saharan Africa (SSA).

Specific Aims:

1. Define the descriptive epidemiology and infectious exposures related to pediatric malignancies in SSA.
2. Conduct transformative inter-disciplinary collaborative research to reduce the burden of KS and lymphoma among children and adolescents in SSA.
3. Support the formation of a multi-national collaborative resource in clinical and translational research, training, and career development for African scientific leaders in HIV and pediatric cancer.

Overlap: None

P42-ES027725 (Moorthy) **New**

02/28/20 – 01/31/25

0.42 CM

NIH

Project Title: SUPERFUND: Polycyclic Aromatic Hydrocarbons: Ultrasensitive Detection, Early Life Exposures-Clinical Outcomes (Preterm Births, Chronic Lung Disease, and Neurocognitive Deficits), Prevention and Remediation

Contact: James R. Williams; Email: williamsjr@nieh.nih.gov; Phone:

Role: Core Leader

Major Goals: The over-arching hypothesis of the BCM-Rice SRP is that early life exposure to PAHs, which are present in superfund sites, increases the risk of PTBs and incrementally augments major morbidities such as BPD and neurocognitive deficits.

Specific Aims:

1. To develop ultrasensitive detection and identification strategies (e.g., surface-enhanced Raman Spectra (SERA) and surface-enhanced Infrared Absorption spectroscopy (SEIRA) for primary and secondary PAH-based compounds in air, water, and soil based on optically active engineered nanomaterials (project 1).
2. To determine molecular mechanisms by which early life exposure to PAH mixtures increases the risks for preterm births, which in turn leads to BPD and neurocognitive deficits.
3. To develop novel remediation technologies to treat sediments contaminated with PAHs in a manner that completely removes the health risks while adding value to the impacted media.

4. To develop novel strategies to prevent and reduce the health burden associated with PAHs present in superfund sites through our Community.

Overlap: None

P30CA125123 (Heslop) **New**

07/01/2020 – 06/30/2025

0.85 CM

NIH/NCI

Project Title: Baylor College of Medicine Cancer Center: Biostatistics and Data Management Core

Contact: Funmi Elesinmogun; email: elesinmf@mail.nih.gov

Role: Quantitative Science Shared Resource and QSSR Core Leader

Major Goals: The overall goal is to unify and organize strong, independent scientific programs into a well-functioning Cancer Center.

Specific Aims:

1. To facilitate collaborative multidisciplinary research and patient care among our Members from different Departments and Centers by establishing Integrated Research Programs.
2. To translate our basic discoveries to the clinic to impact morbidity and mortality from cancer in our Catchment Area and globally.
3. To provide infrastructure support for the conduct of our basic, translational, clinical, and population science research.
4. To promote implementation science research and outreach programs through Community Outreach and Engagement (COE) to reduce the cancer burden in our CA.
5. To establish an effective cancer education program (CRCE) that is integrated with our research to spawn the next generation of scientists and physicians.

Overlap: None

U01-HG006485 (Plon) **New**

08/01/2020 – 05/31/2022

0.54 CM NIH

Project Title: (MPI) Evaluating Utility and Improving Implementation of Genomic Sequencing for Pediatric Cancer Patients in the Diverse Population and Healthcare Setting of Texas: The KIDSCANSEQ Study

Contact: Zephuan Harvey; Email: harveyz@mail.nih.gov

Through this Clinical Sequencing Evidence-Generating Research (CSER2) with Enhanced Diversity project we will complete a trial (The Texas KidsCanSeq Study) comparing the results of targeted cancer panel sequencing versus genome-scale testing in pediatric cancer patients across diverse clinical settings

Role: Co-Investigator

Major Goals: The goal of the project is to determine the relative utility of more focused panel testing versus genome-scale testing including exome and RNA sequencing in the care of the childhood cancer patients across the diverse Texas population.

Specific Aims:

1. We will assess clinical utility of these tests by measuring the frequency of diagnostic and/or actionable germline and tumor findings and the effect on treatment decisions
2. We will compare uptake by first degree relatives for familial genetic testing and recommended cancer surveillance by race, ethnicity and clinical settings
3. We will describe perceived utility (clinical, psychological, and pragmatic) by surveying and interviewing parents and participating pediatric oncologists
4. We will create and evaluate the use of culturally sensitive educational materials, including videos in English and Spanish, improved integrated genomic test reports and counseling materials, and will compare in-person versus telemedicine exome results disclosure

Overlap: None

R01CA250905 (Rosenberg) **New**

07/01/2020 – 06/30/2025

0.00 CM

NIH

Project Title: Mechanisms of Endogenous DNA Damage Promotion

Contact: Funmi Elesinmogun; Email: elesinmf@mail.nih.gov

Role: Significant Contributor

Major Goals: This project uses the E. coli model to uncover both mechanisms that generate spontaneous endogenous DNA damage, and the functions and mechanisms of DNA-damage promotion by bacterial and human DDPs, to understand how many cancer-promoting proteins drive cancers, which may potentially enable universal, economical cancer/disease screening, and novel anti-cancer drug targets.

Specific Aims:

1. Mechanisms of endogenous DNA-damage promotion by dysregulated E. Coli DDPs.
2. Mechanistic studies of DNA-damage promotion by dysregulated human DNMT1, TFs, and others.
3. Connecting DDP activities to mutation mechanisms and cancer signatures.

Overlap: None

1R21NS123589 (Rao) **New**

07/01/2021 – 02/28/2023

0.24 CM NIH

Project Title: Laser Interstitial Thermal Therapy for the Treatment of Glioblastoma

Contact: Jeannette Gordon; Email: jeannette.gordon@nih.gov

Role: Co-Investigator

Major Goals: Determine the effects on the brain tumor microenvironment after treatment with laser interstitial ther-mal therapy. And to determine how laser interstitial thermal therapy can augment the efficacy of chemotherapy-laden na-noparticles for the treatment of intracranial tumors

Specific Aims:

1. Determine the longitudinal effects on the tumor microenvironment after LITT examining treated mice for the influx of immune cells and genetic changes using NanoString.
2. Determine the efficacy of thermally-released doxorubicin from nano-particles on improving survival in tumor bearing mice.

Overlap: None

P01-AG066606 (Zheng) **New**

06/01/2021 – 02/28/2026

0.24 CM NIH

Project Title: Lysosome Regulation and Signaling in Aging and Alzheimer's Disease

Contact: Jermain Cooper; Email: jermain.cooper@nih.gov

Role: Co-Investigator

Major Goals: The overarching goal of this Program Project Grant application is to investigate the lysosome-to-nucleus signaling pathways regulating lysosomal homeostasis in aging and AD, with a focus on tau pathology. Our central hypothesis is that the lysosome is an integrative sensor of cellular stress capable of nuanced communication with the nucleus to activate a range of transcriptional programs that are governed through unique post-translational modifications of signaling molecules and chromatin structural elements.

Specific Aims:

1. Determine how lysosomal properties change as a function of aging and tauopathy, and upon modification of TFEB or TMEM106B.
2. Quantify the impact of aging and tauopathy on PTM patterns of TFEB and TMEM106B.
3. Define how PTM changes regulate lysosome-to-nucleus signaling and target activation.

Overlap: None

RSG-21-182-01-CDP (Sandulache) **New**

01/01/2022 – 12/31/2025

0.36 CM

American Cancer Society

Project Title: Determining the Impact of Ancestry on Oropharyngeal Cancer Biology and Treatment Response

Contact: Lynne Elmore; Email: lynne.elmore@cancer.org

Role: Co-Investigator

Major Goals: Appropriate staging and treatment paradigm development for OPSCC patients with smoking histories mandates a better understanding of 1) how tobacco exposure interacts with HPV status to modulate

prognosis and 2) identification of biological mechanisms leading to decreased treatment response and poor prognosis in HPV+ smokers.

Specific Aims: 1) how tobacco exposure interacts with HPV status to modulate prognosis and 2) identification of biological mechanisms leading to decreased treatment response and poor prognosis in HPV+ smokers.

Overlap: None

R01 CA271498 (Li/Zhang) **New** 03/14/2022 – 03/13/2027 0.48 CM NIH

Project Title: Next Generation Rat Models of ER+ Breast Cancer

Contact: Shakeeya Mone Eaddy; Email: shakeeya.eaddy@nih.gov

Role: Co-Investigator

Major Goals: The major goal of this proposal is to develop and credential rat models of ER+ breast cancer for studying ER+ breast cancer progression, metastasis and therapeutic resistance.

Specific Aims: (1) To characterize early progression of ER+ BCa in RIIM models.

(2) To characterize the metastatic behaviors of ER+ BCa in RIIM models. (3) To credential ER+ RIIM models in recapitulating therapeutic responses

Overlap: None

W81XWH-21-1-0119 (Foulds) This award 03/15/21 – 03/14/24 0.24 CM

Department of Defense

Project Title: Proteogenomic approaches for finding therapeutic vulnerabilities to treat breast tumors expressing transcriptionally active ESR1 fusions.

Contact: Jamie A. Shortall – Jamie.a.shortall.civ@mail.mil

Role: Co-Investigator

Major goals: Our proposed research will promote the ability to diagnose active *ESR1* fusions accurately in the clinic (by developing an “active *ESR1* gene fusion signature”) and will reveal specific kinase and coactivator inhibitor- based therapeutic vulnerabilities for the treatment of *ESR1* fusion-driven metastatic breast cancer. Our pre-clinical therapeutic data will support the development of future clinical trials for patients expressing active *ESR1* fusions that cannot be treated by existing endocrine therapies.

Specific Aims:

Aim 1: To establish functional rules that predict which ESR1 fusions are active drivers of drug resistance and metastasis

Aim 2: To investigate ESR1 fusion-activated downstream kinases for therapeutic targeting

Aim 3: To identify common coactivators recruited by ESR1 fusions as therapeutic targets

W81XWH-21-1-0107 (Lim) **New** 03/15/21 – 03/14/24 0.66 CM

Department of Defense

Project Title: BC200589P1 - Therapeutic Targeting of Nuclear Hormone Receptors in Neurofibromin/NF1-Depleted Breast Cancer

Contact: Amanda Carrera – amanda.c.carrera.civ@mail.mil

Role: Co-Investigator

Major goals: The overall objective of this project is to investigate this hypothesis in order to assess what therapeutic opportunities are associated with neurofibromin loss in patients

Specific Aims:

Aim 1: We will perform molecular biology experiments to determine whether neurofibromin-loss causes AR activation in both ER+ and ER breast cancer cells, agreeing with its role as an AR repressor.

Aim 2: we will determine whether ER+ NF1 breast cancer is more sensitive to an AR-activating agent SARM, while ER NF1+ breast cancer is more sensitive to an anti-AR agent (enzalutamide), and finally whether adding a MEKi can greatly increase treatment efficacies.

Project Title: OPTIMISE: A Shared Care Approach for Improving Comprehensive Care of Cancer Patients with Comorbidities in A Safety-Net System

Contact: Funmi Elesinmogun; elesinmf@mail.nih.gov

Role: Co-Investigator

Major Goals: This proposal provides strong evidence that *ESR1* translocation events are an emerging class of recurrent somatic mutations that lead to not only therapeutic drug resistance but also lethal metastasis in a subset of patients with ER+ breast cancer, a disease not previously considered to be driven by gene fusions. *These ESR1 fusions cannot be treated with the current standard-of-care ET, as they produce proteins lacking the ERα LBD.* However, we will define gene expression patterns that classify pathogenic *ESR1* fusion proteins and their downstream activated kinases to allow new therapeutics to be developed to treat *ESR1* translocated breast tumors. Results of this study will also shed new mechanistic insight into how ER+ breast cancer becomes ET-resistant and metastatic, a lethal process that is still poorly understood.

R01 CA258040 (Badr) **New**

06/01/2021 – 05/31/2026

0.60 CM NIH

Project Title: Next Generation Rat Models of ER+ Breast Cancer

Contact: Sundaresan Venkatachalam; sundarv@nih.gov

Role: Co-Investigator

Major Goals: Patients receiving UMC will receive their cancer treatment, as directed by their oncologist, a survivorship care plan (SCP) at the end of active treatment, and surveillance visits with their oncologist based on national guidelines. Patients in OPTIMISE will 1) have an oncology nurse navigator assigned to their care team at diagnosis to facilitate oncologist-PCP communication and continuity of care; 2) receive coordinated care between their oncologist and PCP throughout cancer treatment.

Specific Aims: AIM 1 evaluates the impact of OPTIMISE on patient chronic disease self-management (primary outcome) and quality of life (secondary outcome). Aim 2 explores the effects of OPTIMISE on healthcare use and patient unmet needs during and after active cancer treatment. Aim 3 examines the effects of OPTIMISE on oncologist and PCP attitudes and coordination of care. Aim 4 seeks to elucidate patient- and system-level factors that may influence implementation outcomes.

Overlap: None

R01 CACA252685 (Ripley) **New**

08/01/2021 – 07/31/2023

0.12 CM NIH

Project Title: Dynamic BH3 **Profiling** with Patient Derived Organoids of Esophageal Cancer and Mesothelioma Enable Precision-Based Targeting of the Mitochondrial Apoptotic Pathway

Contact: Shakeeya Mone Eaddy; Email: shakeeya.eaddy@nih.gov

Role: Co-Investigator

Major Goals: Our goal is to disrupt the mitochondrial balance that enables carcinogenesis but blocks apoptosis by directly targeting the proteins responsible for resistance.

Specific Aims: Aim 1 We will establish a biochemical toolkit to predict treatment response in esophageal cancer and mesothelioma by utilizing a DBP-PDO model.

Overlap: None

VAMC Assignment Agreement (Yao) **New**

08/01/2021 – 07/31/2023

Michael E. DeBakey VA Medical Center

Project Title: VA-Stratification of Pan**creatic** Cancer Subpopulation for Effective Immunotherapy 0.24 CM

Contact: Qizhi Yao; qizhiyao@bcm.edu

Role: Co-Investigator

Major Goals: The major goal of this project is to determine and characterize PDAC subtypes that are sensitive to combination immunotherapy using MSLN VLPs vaccine plus immune checkpoint inhibitor in preclinical animal models.

Specific Aims: Aim 1: Determine whether the immunogenic subtype of PDAC is responsive to MSLN-VLP vaccine in PDX-hu-NSG model. Aim 2: Determine whether combination therapy with anti-PD-1 Ab enhances MSLN-VLP vaccine responses and efficacy in onco-humice model

Overlap: None

Pending

U24-CA271076 (Zhang)

09/01/2022 – 03/31/2027

0.24 CM

NIH

Project Title: iPGDAC, An Integrative Proteogenomic Data Analysis Center for CPTAC

Contact:

Role: Co-Investigator

Major Goals: To analyze CPTAC proteogenomic data to reveal biological and clinical insights.

Specific Aims: (2) To advance data analysis through computational tool development. (3): To identify candidate biomarkers for targeted protein assays.

Overlap: None

R01 CA262437 (Yun)

04/01/2022 – 03/31/2027

0.24 CM

NIH

Project Title: Role of SORD in Sugar-Mediated Cancer Metastasis

Contact:

Role: Co-Investigator

Major Goals: The major aims of this proposal are 1) Determine how SORD accelerates Colorectal cancer (CRC) motility in the HFCS condition.

Specific Aims: 2) Determine how SORD increases CRC motility in the high-glucose condition. 3) Understand the role of SORD in CRC metastasis in vivo.

Overlap: None

COMPLETED IN LAST 5 YEARS

R01CA207270 (Fuqua)

03/07/17 - 02/28/22

0.24 CM

NIH

Project Title: Mechanisms of AR-ER Collaboration In Hormone Resistance and Metastasis of Breast Cancer

Contact: Barbara Hodgkins; Email: barb.hodgkins@nih.gov; Phone: 240-276-6294

Role: Co-Investigator

Specific Aims:

1. To determine if receptor crosstalk (WT or mutant ER with AR) is a mechanism of resistance to hormonal agents.

2. To determine whether AR employs novel nuclear receptor interactions to drive hormone resistance.

3. To determine AR and ESR1 mutant effects on invasion and metastasis.

Overlap: None

R01CA205594 (Li)

02/01/17 - 01/31/22

0.36 CM NIH

Project Title: Ruxolitinib for Preventing Breast Cancer in Women on Neuroleptics

Contact: Viviana Knowles, viviana.knowles@nih.gov, (240)276-5157

Role: Co-Investigator

The overall goal of this proposal is to test a novel concept in breast cancer prevention for women on antipsychotic dopamine antagonists.

Specific Aims:

1. To determine whether in rodent models bearing mammary early lesions, dopamine-antagonizing neuroleptics activate STAT5 and STAT3 in these early lesions, suppress apoptosis, and accelerate progression to cancer.
2. To determine whether in early lesion-bearing rodents on dopamine-antagonizing neuroleptics, genetic ablation of STAT5 and/or STAT3 restores apoptosis in these early lesions and slows the progression to cancer, and to discover the molecular mechanism by which neuroleptic treatment activates STAT3.
3. To determine whether in early lesion-bearing rodents on dopamine-antagonizing neuroleptics, short-term or intermittent treatment to block STAT5/3 activity prevents mammary tumors.

Overlap: None

R01CA204926 (Li)

02/01/17 - 1/31/22

0.36 CM NIH

Project Title: Novel LGR4 Oncogenic Signaling in Breast Cancer Progression and Metastasis

Contact: Viviana Knowles, viviana.knowles@nih.gov,

Role: Co-Investigator

The overall goal of this proposal is to show that LGR4 binds CBL and prevents it from targeting pEGFR for ubiquitin-mediated proteasomal degradation, leading to sustained EGFR signaling, and that this novel LGR4-CBL-EGFR signaling pathway, together with LGR4-stimulated Wnt signaling, promotes BLBC invasion and metastasis.

Specific Aims:

1. To elucidate the novel interaction between LGR4 and CBL in relation to EGFR activation.
2. To establish LGR4 roles in breast cancer metastasis using mouse models and patient-derived xenografts (PDXs).
3. To determine in mouse models, PDX, and human clinical samples whether both EGFR and Wnt signaling play critical roles in breast cancer metastasis stimulated by LGR4.

Overlap: None

CCR185478284 (Roarty)

08/21/18 – 08/20/21

0.12 CM

Susan G Komen

Project Title: WNT Pathway Regulation of Metastatic Progression in TNBC

Contact: ResearchPrograms@komen.org

Role: Co-Investigator

Major Goals: The ultimate goal of the proposed studies will be to discover novel molecular and cellular interactions facilitating the cooperation and plasticity between these cell entities and leverage these findings to render metastatic progression of TNBCs therapeutically vulnerable. Disrupting these cellular interactions by selective targeting of Wnt signaling at key stages of disease, either alone or in combination with other therapies, may block progression and reduce metastatic burden to help improve TNBC patient outcome.

Overlap: None

RP170172 (Rosen)

08/31/20 -08/31/21 (NCE)

0.24 CM

CPRIT

Project Title: Targeting Therapy Resistance using Epithelial to Mesenchymal Transition (EMT) Pathways in Preclinical Claudin Low Breast Cancer Models

Role: Co-Investigator

The overall goal of this project is to have a significant impact on therapy for triple-negative and claudin-low breast cancers as well as potentially other solid cancers, such as pancreatic and colon cancer that have TICs as potential crucial cells involved in both drug resistance as well as metastatic capacity. Competitive renewal of previous CPRIT grant.

Specific Aims:

1. To identify the role of EMT in breast cancer response to chemotherapy in both the primary and metastatic setting using unique basal and claudin-low mouse and human breast cancer models and the Z-cad sensor to detect dynamic transitions between the epithelial and mesenchymal states.
2. To elucidate the response of primary and metastatic breast cancer to epigenetic and other targeted therapies that promote the EMT to MET transition.
3. To determine the efficacy of these therapies, which alter the EMT status of breast cancer in enhancing the response to adjuvant chemotherapy treatment in both the primary and metastatic setting with an emphasis on the treatment of established metastatic disease.

Overlap: None

Cancer Prog M-2018 (Rosen) 04/01/18 – 03/31/21 0.12 CM

Adrienne Helis Malvin Medical Research Foundation

Project Title: Overcoming the Roadblocks for Breast Cancer Immunotherapy

Role: Co-Investigator

Specific Aims:

1. Determine if TIN functionally mediate de novo and acquired resistance against immunotherapies including ICBT and CAR-T.

2: Determine if ED and/or anti-Jag1 treatment increases the efficacy of ICBT and CAR-T in TIN-enriched, ERmammary tumor models, Mammary tumor models.

Overlap: None

BCRF-19-055 (Fuqua) 10/01/18 – 0.36 CM

09/30/20 Breast Cancer Research Foundation

Project Title: Role of ESR1 mutations in breast cancer progression

Contact: Margaret Flowers; Email: mflowers@bcrfcure.org; Phone: 646-497-2611

Role: Co-Investigator

Specific Aims:

1) To conduct sensitive retrospective ESR1 mutation sequence detection and quantitation using an established digital drop BioRad QX100 PCR assay to determine ESR1 mutation frequency, and address whether ESR1 mutations confer resistance to select hormonal therapies

2) To evaluate subclonal evolution and metastatic dissemination of ESR1 mutants, and determine whether a dominant ESR1 mutation phenotype drives distant metastasis.

3) To identify and validate biologic targets activated by ESR1 mutations by identifying paracrine mechanisms, such as secretion of cytokines and growth factors, and genomic changes using ChIPSeq coupled with RNASeq technologies on stable mutant-expressing clones

Overlap: None

U01CA214172 (Paulovich/Lewis) 09/19/16 - 08/31/21 0.21 CM NIH

Project Title: (MPI) A Unique Approach Combining Avatar Mice and Targeted Mass Spectrometry to Identify Blood Biomarkers For Early Detection of Breast Cancer

Contact: Grants Management Specialist: Justin Birken Email: birkenjg@mail.nih.gov Phone: 240-276-6300 Role: Co-Investigator

Major Goals: The major goal of this grant is to identify plasma biomarkers that could be used in conjunction with mammography ($\pm$ ultrasound) to improve the sensitivity and specificity of breast cancer screening programs, facilitating early detection and thereby reducing breast cancer mortality.

Overlap: None

W81XWH-18-1-0040 (Kavuri) 02/15/18 - 02/14/21 0.24 CM

Department of Defense, Level 2

Project Title: Targeting DDR1 Aberrations in Metastatic ER+ Breast Cancer

Grant Contact: Nicholas E. Simon, nicholas.e.simon2.ctr@mail.mil

Role: Co-Investigator

Major Goals: The AIMS of this project are to 1) Determine the effect of DDR1 overexpression on metastasis in ER+ in vivo models; 2) Determine the functional impact of DDR1 somatic mutations on tumor growth and metastasis in ER+ in vitro and in vivo models, and 3) Establish a strategy to effectively treat DDR1 overexpressed and activating mutant breast cancers.

Specific Aims:

1. Determine the effect of DDR1 overexpression on metastasis in ER+ in vivo models.
2. Determine the functional impact of DDR1 somatic mutations on tumor growth and metastasis in ER+ in vitro and in vivo models.
3. Establish a strategy to effectively treat DDR1 overexpressed and activating mutant breast cancers.

Overlap: None

U01HG006485 (Plon)

08/01/12 - 05/31/21

0.60 CM

NHGRI

Project Title: Incorporation of Genomic Sequencing into Pediatric Cancer Care

Contact: Lucia Hindorff; E-mail: hindorffl@mail.nih.gov

Role: Co-Investigator

Major Goals: The goal of this Exploratory Clinical Sequencing project is to integrate CLIA-certified germline and tumor exome sequencing information generated by the Whole Genome Laboratory into the care of childhood cancer patients with high-risk solid tumors and brain tumors of the Texas Children's Cancer Center.

Overlap: None

(Stevens)

07/01/18 - 06/30/19

0.24CM Cure

Childhood Cancer

Project Title: A Trial of Atovaquone with Conventional Chemotherapy for Pediatric AML (Atacc Aml)

Role: Co-Investigator

Major Goals: In this project we hope to develop a multi-institutional clinical trial that will test atovaquone - a well-tolerated agent in combination with traditional cytotoxic chemotherapy for pediatric AML.

Overlap: None

CCR16380599 (Kavuri)

09/15/17 - 09/14/19

0.24CM

Susan. G. Komen Career Catalyst award

Project Title: Career Catalyst - Kinome Analysis to Rationalize Targeting HER2 and DDR1 Breast Cancer Mutations

Grant Contact: Jamie Stanford – jstanford@komen.org

Role: Co-Investigator

Major Goals: The main goal of this project is to understand resistance to HER2-targeted therapy arising through kinome reprogramming

Specific Aims:

1. Aim-1. Determine kinome reprogramming in response to HER2-targeted drugs in HER2-amplified breast cancer PDX models.
2. Aim-2. Determine kinome reprogramming in response to HER2 oncogenic mutations in the context of endocrine therapy resistance in ER+ breast cancers.
3. Aim-3. Determine the impact of HER2-targeted drugs on “phospho-HER2-enriched” basal-like subtype breast cancer models.

Overlap: None

(Lerner)

08/15/17 - 08/14/19

0.12CM

Bladder Cancer Advocacy Network (BCAN)

Proteogenomic characterization of muscle invasive bladder cancer to identify mechanisms of resistance and targets for therapy

The aims are genomic and proteomic characterization of pre-treatment muscle invasive bladder tumors to determine profiles associated with treatment response and resistance and to validate therapeutic compounds derived from the discovery based proteogenomic studies in Aim 1 in PDX models and in naturally occurring bladder cancer in pet dogs.

Role: Co-Investigator

Overlap: None

W81XWH-16-1-0620 (Li)

09/15/16 - 09/14/19

0.36CM

DOD

Project Title: Beyond Apoptosis, Bcl-xL in Breast Cancer Metastasis

Contact: Grants administrator: Chris Meinberg,

Role: Co-Investigator

Major Goals: The overall goal of this proposal to determine the mechanisms by which Bcl-xL promotes migration and invasion in breast cancer cell lines and mouse models, and explore targeting Bcl-xL in preventing and treating breast cancer metastasis in mouse models and PDX models.

Aims

- 1) To investigate molecular mechanisms by which Bcl-xL promotes breast cancer metastasis using breast cancer cell lines
- 2) To establish whether nuclear Bcl-xL drives breast cancer metastasis in vivo using cell line xenografts, genetically engineered mouse models, and PDXs
- 3) To determine whether full suppression of Bcl-xL or blockade of nuclear roles of Bcl-xL prevent or treat metastatic breast cancer in mouse models

Overlap: None

R01CA072038 (Fuqua)

09/01/14 - 08/31/19

0.36CM

NIH

Project Title: Integration of Predictive Biomarkers of Hormone Resistance in Breast Cancer

Contact: Rosemary Ward; Email: wardros@mail.nih.gov; Phone: 301-496-3182

Role: Co-Investigator

Specific Aims:

- 1) To identify and validate new clinical targets of resistance to antiestrogens, such as tamoxifen (Tam) and aromatase inhibitors (AIs)
- 2) To integrate the classification of resistance targets and determine how they impact on ER α function.
- 3) To target resistance mechanisms to prevent the development of resistance and to restore hormone sensitivity, and validate selective candidates for their ability to predict resistance to Tam.

Overlap: None

BCRF-18-055 (Fuqua)

10/01/14 – 09/30/19

0.36CM

Breast Cancer Research Foundation

Project Title: Role of ESR1 mutations in breast cancer progression

Contact: Margaret Flowers; Email: mflowers@bcrcure.org; Phone: 646-497-2611

Role: Co-Investigator

Specific Aims:

- 1) To conduct sensitive retrospective ESR1 mutation sequence detection and quantitation using an established digital drop BioRad QX100 PCR assay to determine ESR1 mutation frequency, and address whether ESR1 mutations confer resistance to select hormonal therapies
- 2) To evaluate subclonal evolution and metastatic dissemination of ESR1 mutants, and determine whether a dominant ESR1 mutation phenotype drives distant metastasis.

3) To identify and validate biologic targets activated by ESR1 mutations by identifying paracrine mechanisms, such as secretion of cytokines and growth factors, and genomic changes using ChIPSeq coupled with RNASeq technologies on stable mutant-expressing clones

Overlap: None

KIM, BEOM-JUN

Active

U01CA214125 (Ellis) **Reduced effort**

06/01/17-05/31/2022

3.0 CM

NIH

Project Title: (MPI) Microscaled Proteogenomics for Cancer Clinical Trials (CPTAC)

Contact: Viviana Knowles; Email: Viviana.Knowles@nih.gov;

Role: Instructor

Major Goals: The overall goal of our proposal leverages state-of-the-art quantitative discovery proteomics and phosphoproteomics as well as targeted assays to measure the kinome and chromatin modifications. These sensitive and reproducible pipelines will be used to analyze preclinical models, well-annotated cohorts and clinical trial samples in an iterative design. A robust proteogenomics pipeline developed by our group will be used to analyze and visualize the data.

Specific Aims

1. Determine the adequacy of our microscaled proteomic pipelines and PDX resources in revealing tumor biology.
2. Develop a prioritization scheme for clinical trial sample analysis using Tier 2 (core needle biopsies from small-scale neoadjuvant studies samples)
3. Determine interactions between the proteogenome, drug response and outcomes in breast cancer neoadjuvant clinical trials.

U54CA233223 (Mitsiades)

09/20/18 – 06/30/23

2.4CM NIH/

NCI

Project Title: Minority PDX Development and Trial Center: Baylor College of Medicine and MD Anderson Cancer Center Collaboration in Mechanistic Studies to Dissect and Combat Health Disparities in Cancer

RP2: Targeting Estrogen receptor and DNA damage repair disparities in African American and Hispanic/Latino breast cancer using Patient- Derived Breast Cancer Xenografts

Grant Contact: Ashley Salo - ashley.salo@nih.gov

Role: Instructor

Major Goals: The objective of this study is to characterize the genome, transcriptome and kinome of 100 breast cancer PDX lines, >70% of which are derived from patients of African American or Hispanic/Latino ethnicity. By understanding fundamental cancer pathways specific to these minority groups this study will delineate efficacy of pre-existing, CTEP-approved therapies in minority ethnicities.

Specific Aims:

1. Systematically characterize estrogen receptor (ER) signaling and response to endocrine treatment in PDX lines derived from AA and Hispanic breast cancer patients.
2. Characterize DNA repair profiles of ER+ and triple negative PDX lines across ethnic groups.
3. Generate -omics' based network profiles specific for Hispanic, AA and CA PDXs.

P50CA186784 (Ellis) **New**

08/01/20 – 07/31/25

2.4 CM

NIH/NCI

Project Title: Translational Research in Breast Cancer

Grant Contact: Funmi Elesinmogun - elesinmf@mail.nih.gov

Project 1: Basic Co-Leader – 1.20 CM

The objectives of this project are thus to identify treatment-resistance drivers in ER+ breast cancer and to target their therapeutic vulnerabilities.

Project 2: Basic Co-Leader – 1.20 CM

Major Goals: Our proposal aims to develop new strategies to combat a common and aggressive subtype of human breast cancer (triple-negative breast cancer, or TNBC). We propose to evaluate the efficacy of sitravatinib in metastatic TNBC patients, delineate which standard of care agents are best to combine with

sitravatinib, and develop new ways to capitalize on the anti-tumoral and pro-immune effects of sitravatinib in the context of immune checkpoint therapies.

Adrienne Helis Malvin Medical Research Foundation (Foulds) NEW 10/01/20 – 09/30/23 0.60 CM

Kinase inhibition for ESR1 fusion-driven breast cancer

Role: Co-Investigator

Major Goals: This proposal provides strong evidence that ESR1 translocation events are an emerging class of recurrent somatic mutations that lead to not only therapeutic drug resistance but also lethal metastasis in a subset of patients with ER+ breast cancer, a disease not previously considered to be driven by gene fusions. These ESR1 fusions cannot be treated with the current standard-of-care ET, as they produce proteins lacking the ER α LBD. However, we will define gene expression patterns that classify pathogenic ESR1 fusion proteins and their downstream activated kinases to allow new therapeutics to be developed to treat ESR1 translocated breast tumors. Results of this study will also shed new mechanistic insight into how ER+ breast cancer becomes ET-resistant and metastatic, a lethal process that is still poorly understood.

W81XWH-21-1-0119 (Foulds) This Award

03/15/21 – 03/14/24

1.2 CM

Department of Defense

Project Title: Proteogenomic approaches for finding therapeutic vulnerabilities to treat breast tumors expressing transcriptionally active ESR1 fusions.

Contact: Jamie A. Shortall – Jamie.a.shortall.civ@mail.mil

Role: Co-Investigator

Major goals: Our proposed research will promote the ability to diagnose active *ESR1* fusions accurately in the clinic (by developing an “active *ESR1* gene fusion signature”) and will reveal specific kinase and coactivator inhibitor- based therapeutic vulnerabilities for the treatment of *ESR1* fusion-driven metastatic breast cancer. Our pre-clinical therapeutic data will support the development of future clinical trials for patients expressing active *ESR1* fusions that cannot be treated by existing endocrine therapies.

Specific Aims:

Aim 1: To establish functional rules that predict which ESR1 fusions are active drivers of drug resistance and metastasis

Aim 2: To investigate ESR1 fusion-activated downstream kinases for therapeutic targeting

Aim 3: To identify common coactivators recruited by ESR1 fusions as therapeutic targets

W81XWH-21-1-0634 (Lim) New

08/01/21 – 07/31/23

2.4 CM

Department of Defense

Project Title: Optimizing Treatment for NF1-Deficient Metastatic ER+ Breast Cancers (Expansion Award)

Role: Co-Investigator

Major Goals: The major goal is to further interrogate approaches to diagnose NF1 loss in clinical samples in order to expand eligibility criteria for trials targeting ER+ breast cancer with NF1 loss.

Specific Aims

1. we will further assess whether this MS-based diagnostic approach can adequately assess NF1 protein levels in patients who will be selected first by DNA sequencing

2. we will functionally characterize these NF1 mutants to assess whether they should be included in future trials because, despite being mis-sense they disrupt NF1 function in a manner that sensitizes to the fulvestrant/binimetinib combination.

PENDING

None

COMPLETED IN LAST 5 YEARS

RR140033 (Ellis)

06/01/14-05/31/2020 NCE

2.1CM

CPRIT

Established Investigator Recruitment Award

Project Title: Proteogenomic and genomic analysis of luminal and triple negative breast cancer for targeted therapeutics discovery.

Contact: Michael Brown; Email: mbrown@cprit.state.tx.us

Role: Instructor

Specific Aims

1. Develop a mechanism-based classification of endocrine therapy resistant ER+ HER2- breast cancer and translate these findings into improved clinical outcomes through clinical trials in the neoadjuvant and metastatic settings
2. Develop a mechanism-based classification of chemotherapy resistant ER- HER2- breast cancer and translate these findings into improved clinical outcomes through clinical trials in the neoadjuvant and metastatic settings
3. Serve goals 1 and 2 through the development of clinically actionable CLIA and/or FDA approved tests based on the measurement of critical DNA, RNA, protein and post-translational modifications that define druggable biology

W81XWH-18-1-0040 (Kavuri)

02/15/18 – 06/30/20

0.6CM

Department of Defense, Level 2

Project Title: Targeting DDR1 Aberrations in Metastatic ER+ Breast Cancer

Grant Contact: Nicholas E. Simon, nicholas.e.simon2.ctr@mail.mil

Role: Instructor

Major Goals: The AIMS of this project are to 1) Determine the effect of DDR1 overexpression on metastasis in ER+ in vivo models; 2) Determine the functional impact of DDR1 somatic mutations on tumor growth and metastasis in ER+ in vitro and in vivo models, and 3) Establish a strategy to effectively treat DDR1 overexpressed and activating mutant breast cancers.

Specific Aims:

1. Determine the effect of DDR1 overexpression on metastasis in ER+ in vivo models.
2. Determine the functional impact of DDR1 somatic mutations on tumor growth and metastasis in ER+ in vitro and in vivo models.
3. Establish a strategy to effectively treat DDR1 overexpressed and activating mutant breast cancers.

Overlap:

None

OTHER SUPPORT

O'MALLEY, BERT W

Current Support

5 P01 DK113954-04 (O'Malley, PI)	07/01/18 – 04/30/23	1.8 calendar
National Institutes of Health/NIDDK	Direct (5yr total)	

Nuclear Receptors and their Coactivators as Mediators of Systems Metabolism

The goal of the research is to elucidate the mechanisms by which transcriptional coactivators direct the metabolic actions of nuclear receptor and transcription factor signaling to impact whole body energy balance.

Admin Core: Direct

Project 3: Direct

Grants Management Specialist: Christina Coriz; corizc@niddk.nih.gov;

2 R01 HD008188-49 (O'Malley, PI)	08/15/19 – 03/31/24	1.8 calendar
National Institutes of Health/NICHD	Direct (5yr total)	

Reproductive Hormones: Biological and Molecular Actions

The major goals of this project are to elucidate the precise molecular mechanisms by which NRs regulate gene expression; to study the conformational alterations in NRs and coactivators that allow formation of an active transcriptional complex; to determine the functional partners of coactivators that impart regulation of (a) transcription initiation, (b) elongation, (c) alternative splicing, and (d) transcription complex turnover/degradation.

5 P30 CA125123-15 (Heslop, PI)	08/14/20 – 06/30/25	0.5 calendar
National Institutes of Health-NCI	salary & fringe only-yr10)	

Baylor College of Medicine Cancer Center

Major Goals of Project: 1) to establish a Cancer Center consisting of a consortium of the College and its primary teaching hospital affiliates (Ben Taub General Hospital, Michael DeBakey Veterans Hospital, Texas Children's Hospital, and St. Luke's Episcopal Hospital) and which has more than \$100M total cancer-related research support including nearly \$40M from NCI; and 2) to provide an organizational structure for the integration and enhancement of research, patient care, education, and outreach activities across all of the affiliated institutions.

Grants Management Specialist: Hasnaa Shafik; shafikh@mail.nih.gov;

R01 HD098059 (Han) New	3/6/20 – 12/31/24	0.6 calendar
NIH/NICHD	Direct (5yr total)	salary only

THE GENOMIC FUNCTION OF ESTROGEN RECEPTOR BETA IN ENDOMETRIOSIS

Major Goals: The major goal of this project is to define how Estrogen Receptor beta directly modulates the gene expression profiles in endometriotic lesions to enhance anti-apoptosis, invasion, and angiogenesis for endometriosis progression.

Grants Management Specialist: Melissa R Copeland; melissa.copeland@nih.gov;

R01 GM143380 (Wang, PI) New	7/8/21 – 4/30/26	0.36 calendar
NIH/NIGMS	Direct (5yr total)	(salary only) Molecular

mechanism of Androgen Receptor mediated transcription

Major Goals: Utilize cryoEM to visualize functional AR-CoR complexes, to determine the structural basis of transcriptionally active AR/V7 complexes. Employs advanced imaging techniques to achieve unprecedented insights into the structure and function of AR-CoR complexes, AR heterodimers, and the drugs that target them.

Grants Management Specialist: Brett Hodgkins, brett.hodgkins@nih.gov,

U54 CA233223 (Mitsiades)	9/20/18 – 6/30/23	0.24 calendar
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NIH/NCI Direct(5yr total) (salary only) Minority
 PDX Development and Trial Center: Baylor College of Medicine and MD Anderson Cancer Center
 Collaboration on Mechanistic Studies to Dissect and Combat Health Disparities in Cancer
 Major Goals: The goals of this grant are to utilize patient derived xenografts of prostate cancer to evaluate novel anti-cancer agents in mouse tumor models, including steroid receptor coactivator inhibitors developed by the O'Malley laboratory.
Grants Management Specialist: Ashley Salo Email: ashley.salo@nih.gov Phone:

No # (Sreekumar, PI) 08/04/17- 08/3/22 0.36 calendar
 Agilent Mass Spectrometry Direct (5yr total) (salary only) Center for
 Excellence
 Major Goals: This grant funds development of non-invasive metabolic markers in serum/plasma and urine for cancer detection and prognosis.
Grants Management Specialist: Sudharshana Seshadiri; [Sudharshana Seshadiri@agilent.com](mailto:Sudharshana.Seshadiri@agilent.com);

1-W81XWH-21-1-0119 / BC200317 (Foulds) **This Award** 3/15/21 – 3/14/24 0.12 calendar
 Department of Defense Direct (3yr total) (salary only)
 Proteogenomic Approaches for Finding Therapeutic Vulnerabilities to Treat Breast Tumors Expressing
 Transcriptionally Active ESR1 Fusions

Major Goals: Our proposed research will promote the ability to diagnose active ESR1 fusions accurately in the clinic (by developing an “active ESR1 gene fusion signature”) and will reveal specific kinase and coactivator inhibitor- based therapeutic vulnerabilities for the treatment of ESR1 fusion-driven metastatic breast cancer. Our pre-clinical therapeutic data will support the development of future clinical trials for patients expressing active ESR1 fusions that cannot be treated by existing endocrine therapies.
Grants Management Specialist: Amanda Carrera ; Amanda.C.Carrera.civ@mail.mil; Phone:

Pending Support

R01 HD007857 (O'Malley, PI) Renewal Pending 09/01/22 – 08/31/27 1.8 calendar
 NIH/NICHD Direct (5yr total)
 Sex Hormone Receptor Component and The Cell Genome
 Major Goals: The major goals of this project are to determine the actions of female sex steroid receptors in differentiated reproductive tissues; to determine the impact of coactivators on reproductive tissue ‘growth’ and pathology; to determine the intracellular mechanisms by which coactivator levels and activities are regulated in target cells of nuclear receptors; & to prove that the mechanisms discovered in cells in culture are operative in living animals.
Grants Management Specialist: Melissa R Copeland; melissa.copeland@nih.gov;

AZ210011 (O'Malley, PI) 06/01/22 – 05/31/25 1.8 calendar
 DOD Direct (5yr total)
 REG γ REGULATION OF BRAIN TAU PROTEIN
 Major Goals: The goals of this grant are to elucidate the link between proteasome-mediated protein degradation of Tau proteins in the brain and the consequences of deficiencies in this pathway that can lead to Alzheimer's disease. In particular, the role of the alternative REG γ proteasome cap is a focus of this grant.
Grants Management Specialist: not yet assigned

R01 AG075657 (O'Malley, PI) 7/1/22 – 6/30/27 1.8 calendar
 NIH/NIA Direct (5yr total)

REGULATION OF NEURONAL TAU PROTEIN BY REG γ

Major Goals: The goals of this grant are to characterize the role of the alternative proteasome cap protein, REG γ in the proteasome-mediated degradation of the Tau protein. The mechanism by which this alternatively-capped proteasome is able to degrade insoluble Tau protein is sought in this proposal.

Grants Management Specialist: not yet assigned

R01 NEW (Wang/Lonard, MPI)
NIH

09/01/22 – 08/31/27
Direct (5yr total)

0.6 calendar
(salary only) Brain

SRC-1 and Alzheimer's Disease

Major Goals: The goal of this project is to understand the role of steroid receptor coactivator 1 (SRC-1) in the support of central nervous system pathways that protect it against Alzheimer's disease. The use of SRC-1 small molecule stimulators to protect the brain from Alzheimer's disease is explored.

Grants Management Specialist: not yet assigned

Overlap:

None

Percent efforts will be adjusted, pending funding notification so that no overlap occurs. Adjustments will be made in the event that funding is awarded on pending studies.

Previous Support

5 R01 HD007857-49/50 (O'Malley, PI) - **Ended**

03/15/17 – 02/28/22

1.8 calendar

National Institutes of Health/NICHD

Direct (2yr total)

Sex Hormone Receptor Components and the Cell Genome

The major goals of this project are to determine the actions of female sex steroid receptors in differentiated reproductive tissues; to determine the impact of coactivators on reproductive tissue 'growth' and pathology; to determine the intracellular mechanisms by which coactivator levels and activities are regulated in target cells of nuclear receptors; & to prove that the mechanisms discovered in cells in culture are operative in living animals.

Grants Management Specialist: Melissa R Copeland; mh601f@nih.gov;

CPRIT #5RP150648-YR05 (O'Malley, PI) **Ended**

06/01/20 – 05/31/21

0.6 calendar

CPRIT - MIRA

Direct (no cost extension)

Admin Core: Steroid receptor coactivator-2 (SRC-2) and GATA2 cooperate with androgen receptor to promote prostate cancer progression and androgen resistance

The main goal of this project is administrative and informatics.

Grants Management Specialist: Sandra Balderrama; www.cprit.state.tx.us;

CPRIT #RP170500-YR03/03 (O'Malley, PI) **Ended**

08/31/17 – 08/30/20

1.2 calendar

CPRIT

Direct NCE

Development of Next Generation Steroid Receptor Coactivator Small Molecule Inhibitors as Novel Agents to Target Therapy-Resistant Breast Cancer

Grants Management Specialist: Sandra Balderrama; www.cprit.state.tx.us;

SRA - COACTIGON (O'Malley, PI)

11/13/17 – 06/30/20

0.6 calendar

Coactigon, Inc.

Direct Commercial

Development of SRC Modulating Small Molecules

Small Molecule Inhibitors of Steroid Receptor Coactivator to Treat Breast Cancer

Clinical and preclinical evidence suggest that overexpression of SRC-3, which is activated by a variety of kinase pathways, plays a special role in breast cancer by augmenting signaling from ER and other TFs, thereby contributing strongly to resistance to endocrine therapies.

**Grants Management Specialist: Steven Buxbaum; Steven.buxbaum@haynesboone.com;
; Fax (713) 236-5404**

4 P01 DK059820 (O'Malley, PI) 07/01/15 – 06/30/18 1.44 calendar
National Institutes of Health/NIDDK (3yr total)
Genetic and Metabolic Fingerprints of Coactivators "Program Project"
Project 1 (0.96 calendar) Goals: focus on the hepatic functions of the AMPK/SRC-2 signaling axis as it pertains to dietary fat absorption and whole body energy accretion.
Administrative Core B (0.48 calendar) Goals: provide centralized scientific and administrative management, web site and database development for data sharing, financial control services, computer/network/server support and maintenance.

**Grants Management Specialist: Diana O'Donovan; o'donovand@extra.niddk.nih.gov;
Fax:**

DAMD W81XWH-13-1-0285 (O'Malley, PI) 08/15/15 – 08/14/18 5.76 calendar
Department of Defense (BC120894) (3yr total)
Development of Coactivator-Dependent, First-in-Class Therapies for Breast Cancer
Goals: to develop small molecule inhibitors designed to inhibit steroid receptor coactivator-3 (SRC-3) for use as breast cancer therapeutic agents.

**Science Officer: Daniel Monson, Ph.D.; Breast Cancer Research Program;
daniel.m.monson.civ@mail.mil**

5 U24 DK097748-02/05 (O'Malley, PI) 09/17/15 – 08/31/17 0.24 calendar
National Institutes of Health/NIDDK (2yr total)
A Hub for the Nuclear Receptor Signaling Atlas
Goals: The Hub will support a public web portal (www.nursa.org) as part of a comprehensive approach to elucidating the role of NRs in normal physiology and disease.
Goals: NURSA will assist in generating important new concepts and validated datasets that will provide the foundation for research leading to a more comprehensive understanding of the role of NRs in human physiology and metabolic disease.

Grants Management Specialist: Todd Le; let@extra.niddk.nih.gov; Fax (301) 480-0475

1P50CA186784-01 (Osborne, PI) 09/19/15 – 08/31/19 0.17 calendar
NIH/NCI (4yr total)
SPORE - Translational Research in Breast Cancer
Project 2: (O'Malley, Project Leader)
Goals: To expedite the translation of basic research into applied clinical applications in breast cancer detection, treatment and prevention.

Grants Management Specialist: Shane Woodward; woodwars@mail.nih.gov;

CASIS GA-2014-136 (Dacso, PI) 05/27/15 – 05/31/19 0.24 calendar
Center for the Advancement of Science in Space (4yr total)
Microgravity as a Stress Accelerator for OMIC Profiling of Human Disease
Goals: 1. Develop an integrated omics platform focused on circadian rhythm, initially in animals, subsequently in humans. 2. Develop integrated mathematic expressions for various omic components. 3. Develop a microfluidic platform the assessment of components described in (2) above.

Grants Management Specialist: Nora Thompson; Nora.Thompson.nasa.gov;

OTHER SUPPORT

WANG, J.

ACTIVE

RP220480 (Wang) **NEW**

03/2022 – 02/2025

2.4 CM

CPRIT

RIP PROTACs as a Novel Immunotherapy Sensitizer to Treat Colorectal Cancer

Major goals: we will develop RIP PROTACs as a novel cancer immunotherapy sensitizer and test its efficacy in multiple colorectal cancer models, including syngeneic and transgenic mouse tumor models. In addition, we will try to identify biomarkers to predict the therapeutic efficacy of the combination therapy.

R01AI32956 (Palzkill) **NEW**

01/2022 – 12/2026

0.6 CM

NIH/NIAD

Role of β -Lactamase Mutations in Antibiotic Resistance

The long-term goal of this project is to understand how the KPC-2 carbapenemase hydrolyzes beta-lactam antibiotics using kinetic, structural and computational methods. In addition, the work will examine the mechanistic basis for resistance to the beta-lactamase inhibitor avibactam for drug-resistant KPC variant enzymes

U01AG068031 **NEW** (Wang, PI; Zheng, MPI)

10/01/2020 – 09/30/25

2.40 CM

NIH/NIA

Grant Program Officer: Jean Yuan Email: yuanx4@csr.nih.gov

Developing Novel Soluble Epoxide Hydrolase Inhibitors for the Treatment of Alzheimer's Disease

The major goals of this project are to develop a small molecule inhibitor to target soluble epoxide hydrolase to treat Alzheimer's disease.

W81XWH-21-1-0119 (Foulds) This Award

03/15/21 – 03/14/24

1.80 CM

Department of Defense

Project Title: Proteogenomic approaches for finding therapeutic vulnerabilities to treat breast tumors expressing transcriptionally active ESR1 fusions.

Contact: Jamie A. Shortall – Jamie.a.shortall.civ@mail.mil

Role: PI

Major goals: Our proposed research will promote the ability to diagnose active *ESR1* fusions accurately in the clinic (by developing an “active *ESR1* gene fusion signature”) and will reveal specific kinase and coactivator inhibitor- based therapeutic vulnerabilities for the treatment of *ESR1* fusion-driven metastatic breast cancer. Our pre-clinical therapeutic data will support the development of future clinical trials for patients expressing active *ESR1* fusions that cannot be treated by existing endocrine therapies.

Specific Aims:

Aim 1: To establish functional rules that predict which ESR1 fusions are active drivers of drug resistance and metastasis

Aim 2: To investigate ESR1 fusion-activated downstream kinases for therapeutic targeting

Aim 3: To identify common coactivators recruited by ESR1 fusions as therapeutic targets

R01CA207701 (Wang, PI) **Reduced effort/NCE**

3/15/2017 – 2/28/2023NCE

0.12 CM

NIH/NCI

Grant Program Officer: Yali Fu Email: fuyali@email.nih.gov

Drugging the Undruggable Coactivators using Small Molecule Inhibitors

The major goals of this project are to develop a small molecule inhibitor to target coactivators.

RF1AG062257 (Wang PI, Zheng PI, M. Wang PI)

9/15/2018 – 6/30/2023

1.2 CM

Grant Program Officer: Yih-Woei Fridell Email: yih-woei.fridell@nih.gov

Targeting A Lipid-Mediated Pro-Longevity Pathway as Alzheimer's Therapy

The major goals of this project are to develop antiaging OEA analogs as Alzheimer's Therapy.

R01 CA250503(Wang, PI; ML Wang PI; Woyach PI) 7/01/2020 – 6/30/2025 1.2 CM

NIH/NCI

Grant Program Officer: Weiwei Chen Email: chenw6@mail.nih.gov

Reversible Covalent BTK Degraders as the Next Generation Targeted Therapy to Treat B-cell Malignancies

The major goals of this project are to develop novel BTK degraders to treat B-cell malignancies.

PENDING

None

In Kind Contribution

Isomerase Therapeutics Ltd provided a natural product to evaluate its biological applications.

Baylor College of Medicine; Houston, Texas

08/02/2021-12/31/2023

OVERLAP

None

COMPLETED

RP160805 (Matzuk, PI)

5/18/2016 – 12/30/2021

Cancer Prevention and Research Institute of Texas

CPRIT Grants Contact: Michael Brown magid@cprit.texas.gov

Preclinical Candidate Discovery Core

This is a core facility grant. We provide chemistry and PKPD expertise to the Center for Drug Discovery.

RP170500 (O'Malley, PI)

08/31/2017 – 08/30/2021

CPRIT

Grant Program Officer: Patty Moore, PhD, Senior Program Manager, pmoore@cprit.texas.gov Development of Next Generation Steroid Receptor Coactivator Small Molecule Inhibitors as Novel Agents to Target Therapy-Resistant Breast Cancer

We provide chemistry expertise to synthesize small molecule inhibitors

R01 GM115622 (Wang, PI)

9/22/2015 – 7/31/2021(NCE)

NIH/NIGMS

Grant Program Officer: Charles G Edmonds Email: edmondsc@nigms.nih.gov

Real-Time Quantitative Imaging of Intracellular Biothiol Dynamics

The major goals of this project are to develop molecular probes for quantitative real-time imaging of glutathione and hydrogen sulfide in living cells and in animals.

SRA (O'Malley, PI)

1/1/2019 – 3/30/2020

Coactigon Inc.

This is an industry sponsored research agreement. We provide chemistry and PKPD expertise to develop novel anticancer therapeutics.

R21CA213535-01, NIH/NCI Wang, Jin (MPI, Lu)

07/01/17 – 11/30/19

NIH/NCI

Grant Program Officer: Yali Fu Email: fuyali@email.nih.gov

Engineering Antibody Drug Conjugates to Target P53-Defective Triple Negative Breast Cancer

The major goals of this project are to develop a novel antibody drug conjugate to treat triple negative breast cancer with hemizygous loss of *TP53/POLR2A*.

18X033, NCI/Leidos Biomedical Research Wang, Jin (PI)

03/13/18 – 11/30/18

Exploratory Study of Ratiometric Imaging of GSH in Live Cells

The major goal of this project is to develop a fixable glutathione probe for cell and tissue imaging.