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

PRINCIPAL INVESTIGATOR: Dr. Eric Chang

CONTRACTING ORGANIZATION: Baylor College of Medicine, Houston, TX

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14. ABSTRACT

This project centers on the NF1/neurofibromin tumor suppressor, which was best known as a GTPase Activating Protein (GAP) that represses Ras activity. We have recently shown that NF1 has a GAP-independent activity by functioning also as a transcriptional co-repressor for estrogen receptor α (ER) in ER^+ breast cancer. ER is structurally closely related to the androgen receptor (AR). In this multi-PI grant, we will investigate the hypothesis that We **hypothesized** that NF1, analogous to its role in ER regulation, is also an AR co-repressor. Our **objective** is to assess this hypothesis to explore and exploit the broader consequences of NF1 loss in breast cancer therapeutics. The first specific aim is to define the interactions between neurofibromin and AR by studying neurofibromin's role as an AR co-repressor. Our results showed that AR and NF1 can physically interact in a ligand-dependent manner. While NF1-silencing enhanced AR-dependent transcriptional activities, NF1 overexpression inhibited it. As a result, NF1-depleted AR^+ cancer cells can grow at suboptimal levels of AR agonists. The second specific aim is to assess how hyperactivated AR due to *NF1* loss impacts the treatment of breast cancer by pre-clinically modeling the effects of AR antagonists or SARMs (selective AR modulators). The results from this project period showed that NF1-depletion can affect the choices of anti-AR agents. Enzalutimde which does not have known agonist activity is a better drug to treat these tumors than bicalutamide. Further NF1 loss actives not only AR but also Ras. In support of this, adding a MEK inhibitor can enhance the efficacy of enzalutamide.

15. SUBJECT TERMS
None listed.

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INTRODUCTION

This project centers on the NF1/neurofibromin tumor suppressor, which was best known as a GTPase Activating Protein (GAP) that represses Ras activity. We have recently shown that NF1 has a GAP-independent activity by functioning also as a transcriptional co-repressor for estrogen receptor α (ER) in ER^+ breast cancer. ER is structurally closely related to the androgen receptor (AR). In this multi-PI grant, we will investigate the hypothesis that We **hypothesized** that NF1, analogous to its role in ER regulation, is also an AR co-repressor. Our **objective** is to assess this hypothesis to explore and exploit the broader consequences of NF1 loss in breast cancer therapeutics. The specific aims are:

AIM 1: To define the interactions between neurofibromin and AR by studying neurofibromin's role as an AR co-repressor (primary responsibility of the initiating PI Eric Chang). We will measure:

(A) Ligand-dependent direct binding between neurofibromin and AR, using purified components.

(B) AR-dependent gene expression and AR recruitment to the chromatin as mediated by neurofibromin, using RNA-seq and ChIP-seq.

(C) AR ligand-mediated recruitment of neurofibrom to the chromatin by ChIP-seq.

AIM 2: To assess how hyperactivated AR due to *NF1* loss impacts the treatment of breast cancer by pre-clinically modeling the effects of AR antagonists or SARMs (primary responsibility of the partnering PI Matthew Ellis/Bora Lim).

(A) Measure *in vitro* cell growth/apoptosis upon neurofibromin-depletion in the presence of a SARM (ER^+ models) or an AR antagonist (ER^- models); if successful, these compounds will be tested in combination with a MEKi (binimetinib).
(B) Measure treatment efficacies using xenograft models, leveraging our collection of several $NF1^-$ and AR^+ PDX models as a prelude to potential clinical trials.

KEYWORDS

AI, Aromatase inhibitor
AR, Androgen receptor.
ARE, AR responsive element.
ChIP, Chromatin immunoprecipitation
Co-IP, co-immunoprecipitattion.
DHT, Dihydrotestosterone
DOX, doxycycline.
E2, estrogen/estradiol
EMT, epithelial to mesenchymal transition
ER, estrogen receptor-α
EREs, Estrogen Response Elements
FS, frameshift
GAP, GTPase Activating Protein
HR, hazard ratio HR
IHC, immunohistochemistry
IIA, Intra-iliac artery
KI, knock-in
KM, Kaplan-Meier
KO, knock-out
MEKi, MEK inhibitor.
NF1, Neurofibromatosis type 1

NS, nonsense
PDX, patient-derived xenograft
SARM, Selective AR modulator
SERM, Selective ER modulator
TCGA, The Cancer Genome Atlas

ACCOMPLISHMENTS

AIM 1: To define the interactions between neurofibromin and AR by studying neurofibromin's role as an AR co-repressor.

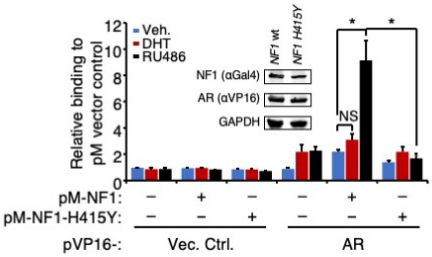

Figure 1. NF1 binds AR in a ligand dependent manner as determined by the mammalian two-hybrid assay. Wild type NF1 or NF1-H415Y mutant fused to the Gal4 activation domain was expressed by the PM vector, while the AR fused to the VP16 transcription activator domain was expressed by the pVP16 vector. These vectors were transduced into 293 cells. The inset is a representative western blot to show all fusion proteins were expressed at comparable levels. AR-NF1 binding turned on the expression of luciferase, which can be measured by bio-illuminance. The 293 cells were treated by either the AR agonist DHT or the AR antagonist RU486. The latter as expected stimulated NF1-AR interaction.

<u>NF1 binds AR in a ligand-dependent manner (Aim 1A)</u>. This project was partially inspired by the finding that a mutation in NF1's nuclear receptor binding domain, HY, was found in prostate cancer. Thus, we investigated in this sub-aim whether NF1 can form a complex with AR and whether this interaction can be weakened by the H415Y mutation. The interactions between a nuclear receptor and co-repressor are induced by an antagonist such as RU486, but not by an agonist such DHT (Dihydrotestosterone). We performed the mammalian two-hybrid assay to examine these interactions and the data agree with our model (Figure 1). That is, while the wild-type NF1 can interact with AR in the presence of RU486, this interaction was greatly reduced with the NF1-H415Y mutant.

Next, we ascertained whether the AR-NF1 interaction can be detected in AR-expressing (AR^+) prostate (LNCaP) and breast cancer cells (MDA-MB-435) by performing co-immunoprecipitation (co-IP). AR co-immunoprecipitated with NF1 in the presence of the RU486 antagonist, but not DHT (Figure 2). MDA-MB-453 breast cancer cells showed similar results (data not shown).

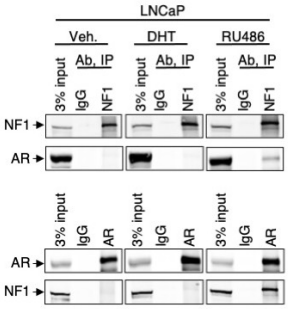

Figure 2. Ligand-dependent co-IP between NF1 and AR in LNCaP cells. LNCaP cells were pre-treated by different AR ligands before IP was performed using antibodies against either AR or NF1, while IgG was used as the antibody control. IP-ed samples were examined by western blot using AR or NF1 antibody to reveal that AR and NF1 can co-IP efficiently in the presence of the AR antagonist RU486. Similar results were obtained after examining MDA-MB-453 cells

Future studies will focus on examining whether the interaction between AR and NF1 is direct using purified components.

<u>NF1-depletion enhances AR transcriptional activities (Aim 1B)</u>. Given our model depicting NF1 as an AR co-repressor, we expect NF1 depletion can increase AR transcriptional activity. Conversely, NF1 overexpression is expected to inhibit AR transcriptional activity. We first investigated this using a luciferase reporter whose activity is under the control of an AR responsive element (ARE). When we silenced *NF1* expression (+DOX) in both LNCaP and MDA-MB-453 cells, ARE-Luc reporter activities increased (Figure 3A). In contrast, when wild type NF1 was overexpressed, ARE-Luc activity decreased (Figure 3B). As a control, we also tested the NF1-H417Y mutant, which as shown above has greatly reduced AR-binding ability, and found that it did not substantially inhibit ARE-Luc activity. To determine whether NF1 levels are also important for affecting expression of endogenous AR-responsive gene, we silenced NF1 expression in LNCaP cells and confirmed that the expression of two well-known AR-dependent genes increased (Figure 3C).

Future studies will examine genome-wide gene expression changes in an unbiased fashion by RNA-seq to further determine AR related pathway changes, and to analyze AR and NF1 recruitment to the chromatin by ChIP.

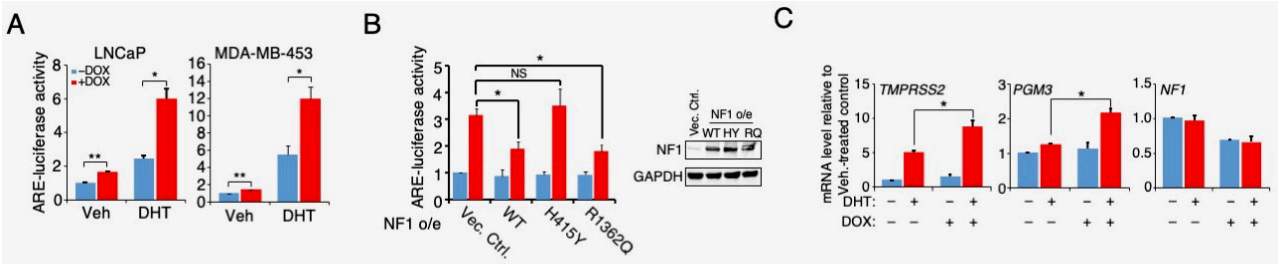

Figure 3. NF1 depletion enhanced while NF1 overexpression repress transcriptional activity of an ARE-luc reporter. A. Prostate cancer LNCaP cells and breast cancer MDA-MB-453 cells carrying the ARE-Luc reporter was transduced by a DOX-inducible shRNA vector to repress *NF1* expression. In both cell lines, *NF1* silencing (+DOX) enhanced the Luc activity, indicating enhancing transcriptional activity at the ARE. **B.** MDA-MB-453 cells carrying the ARE-luc reporter was transduced to express either wild type NF1, the AR-interfering NF1-H415Y mutant, or the GAP-inactivating mutant NF1-R1362Q. Wild type NF1 as expected inhibited ARE activity, while the NF1-H415Y mutant did not. This repression is GAP-independent because the NF1-R1362Q mutant behaved the as as cell lines expressing the wild type NF1. The western blot on the right demonstrated that all tested NF1 proteins were expressed at comparable levels. **C.** NF1 was silenced as in panel-A in LNCaP cells, and mRNA levels of two known AR-dependent genes were measured by qPCR to show that NF1 silencing (right) enhanced the expression of these genes (left).

<u>NF1-depletion induces hypersensitivity to the AR agonist (Aim 1)</u>. Since NF1 loss appears to enhance AR-dependent transcriptional activity, we asked whether NF1-silencing can cause AR^+ cells to be more sensitive to an AR agonist such as DHT. Indeed, the data demonstrated that when NF1 expression was silenced (+DOX), cells can grow better at sub-optimal DHT concentrations as compared to their $NF1^+$ counterparts (Figure 4).

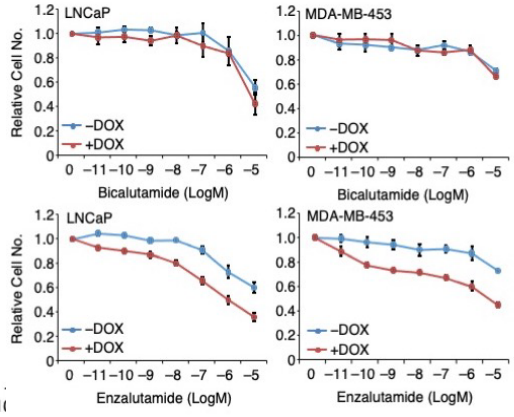

Figure 5. NF1 silencing induced differential responses to anti-AR agents. AR^+ LNCaP and MDA-MB-435 cells carrying the DOX-inducible NF1 shRNA were seeded in the presence of various of concentration of two anti-AR agents, and the cell growth was measured by the MTS assay 7 days later. The data show that NF1-depleted cells are more sensitive to enzalutamide.

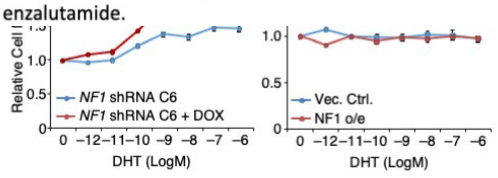

Figure 4. NF1 silencing enhanced sensitivity to AR agonist. AR^+ LNCaP and MDA-MB-435 cells carrying the DOX inducible NF1 shRNA were seeded in the presence of various of concentration of DHT and the cell growth was measured by the MTS assay 7 days later. The AR^- MDA-MB-231 cells were examined as the control which do not respond to DHT. Western blot shows that *NF1* silencing enhanced DHT responsiveness without increasing AR levels.

<u>NF1-depletion affects cellular responses to anti-AR therapeutics (Aim 2A)</u>. As discussed above, we have evidence that NF1-depletion can enhance AR-dependent transcriptional activities. This change in AR signaling may reduce the efficacy of anti-AR agents that have weak agonist activities. To investigate this possibility and to seek a more appropriate drug to treat NF1-depleted AR^+ tumor cells, we treated NF1 wild type or NF1-depleted AR^+ cancer cells with several AR agonists and then measured the impacts on cell growth. Bicalutamide is an AR antagonist with weak agonist activity, and as shown in Figure 5, NF1-silenced AR^+ breast and prostate cancer cells were insensitive to this agent. In contrast, these cells were more sensitive to enzalutamide which mainly blocks AR from entering the nucleus and is not known to have agonist activity.

<u>Adding a MEK inhibitor enhances the treatment efficacy of AR inhibitor, enzalutamide in treating NF1-depleted AR^+ cancer cells (Aim 2A)</u>. We hypothesize that loss of NF1 can turn on not only AR but the Ras-Raf signaling pathways as previously shown in ER^+ breast cancers. Therefore, we postulated that a MEK inhibitor (MEKi) such as binimetinib can block the latter, thereby co-inhibition can more effectively inhibit the NF1-depleted AR^+ cancers. Indeed, our data show that NF1-depleted AR^+ prostate and breast cancer cells are more sensitive to the enzalutamide + binimetinib combination compared to the $NF1^+$ counterparts (Figure 6). Based on this, we will further assess whether blocking another Ras pathway, e.g., that of the PI3K pathway, may more efficiently inhibit or possibly kill NF1-depleted AR^+ cells in the presence of enzalutamide. This will be important for future therapeutic strategy development strategies.

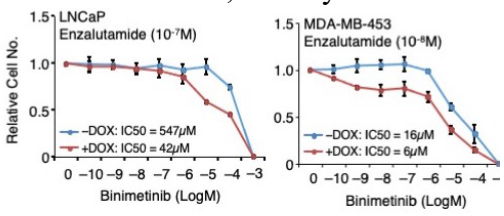

Figure 6. Adding a MEKi enhances enzalutamide efficacies when treating NF1-depleted AR^+ cancer cells. Indicated cells carrying the DOX-inducible shRNA against NF1 were seeded in the presence of a single concentration of enzalutamide plus various concentrations of a MEKi, binimetinib. Cell numbers were assessed by the MTS assay one week later. These data show that NF1-knock down cells are more sensitive to binimetinib.

CHANGES/PROBLEMS

Dr. Matthew Ellis is a physician-scientist who was the original partnering PI on this proposal responsible for therapeutic related studies in Aim 2. He has left Baylor College of Medicine for AstraZeneca where he is now the Senior VP of Early Oncology. We have requested to change the partnering PI to Dr. Bora Lim, who is also a clinician-scientist, and the Director of Translational Research for the Breast Cancer Research Program in the Dan L. Duncan Comprehensive Cancer Center. Dr. Lim is able to assume the 3% Partnering PI effort to provide the clinical expertise/translational input on this project. The Aims of the grant will not change, and she will be able to successfully complete this grant without any increase in funds.

Our project has also been facing many challenges caused by COVID-19, which has led to greatly limited lab access, and animal work was nearly shut down completely. In the next project period, we believe these limitations will be mostly behind us and will allow us to more efficiently conduct experiments requiring animals (Aim 2). NF1 ChIP-seq as proposed in Aim 1 is a challenging experiment due to the fact that NF1's binding to the DNA is through the binding to ER, which directly binds DNA. In addition, we are limited by the toolset of ligands that promote NF1 binding to the DNA. We are in the process of optimizing the cross-linking protocol to increase/stabilize the portion of DNA bound by NF1 to improve the sequencing performance.

PRODUCTS

Clinical Trial: Based on our compelling pre-clinical data, we were able to design a two-cohort phase II study of fulvestrant-binimetinib combination in patients with metastatic ER^+ breast cancers in collaboration with the NCI Combo MATCH program (Protocol #EAY191-N2; Figure 7 for a schema). This trial is approved by NCI Cancer Therapy Evaluation Program (CTEP) as one of the ComboMATCH therapeutic protocols and the final protocol has been developed and submitted to the CTEP during Q1 of 2022. The ComboMATCH is a highly competitive program designed to study rationally combinatorial therapeutics in advanced cancers, based on robust predictive biomarkers-guided selection of the target population.

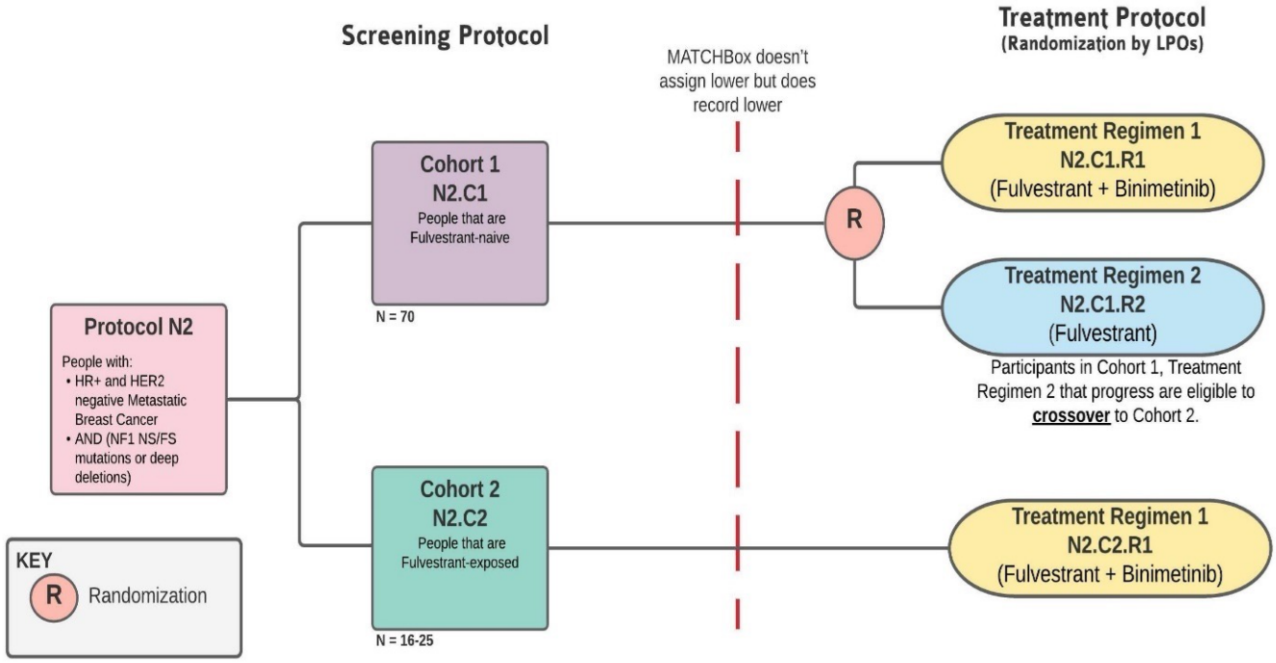

Figure 6. Schema of EAY191-N2
This is a phase II study of binimetinib in combination with fulvestrant in patients with metastatic hormone receptor-positive HER2 negative breast cancers with a non-functional mutation (frameshift or nonsense or genomic deletion) in NF1.
This trial includes two cohorts: the fulvestrant naïve cohort (Cohort 1: randomized) and the fulvestrant resistant cohort (Cohort 2: single arm).

Other Grant awarded with support by this grant:

Our parent grant's partnering PI (Ellis) has submitted an Expansion award to better study other aspects of NF1's properties and got funded. The PI on this grant (Chang) is a co-PI on that grant:

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

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.

Project Number: BC201666/ W81XWH-21-1-0634

Project/Proposal Start and End Date: 09/01/2021-08/31/2023

Total Award Amount (including Indirect Costs):

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name	**Project role**	**ORCID ID**	**Person Mon Worked (Time Period worked 08/01/21-03/14/22)**	**Project contribution**	**Funding support**
Eric Chang	PI	0000-0002-1375-5088	4.2 CM	Design and execute all the studies in this project, and will write the pa-per.	This grant.
Zeyi Zheng	Instructor	0000-0001-6536-4874	1.8 CM	Assist Dr. Chang in the design and execution of all the studies in this project, and supervise Ms. Kenney	This grant.
Hilda Kennedy	Tech	NA	1.8 CM	Provide technical support on all projects.	This grant

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Updated Other Support Attached.

CHANG, ERIC

ACTIVE

W81XWH-19-1-0527 09/01/19 – 08/31/22 (NCE) 1.7 CM (14.17%)***
Department of Defense
Project Title: Direct regulation of estrogen receptor transcription activity by NF1
Grants Contact: Henry Nothnagel- henry.j.nothnagel.civ@mail.mil
Role: Principal Investigator
Major Goals: The objective of this project is to define NF1's role in bone metastasis in order to establish a strategy to stop it
Specific Aims

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.
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 metas-tasis in ER^+ $NF1^-$ cancer.

*** The effort of DOD W81XWH-19-1-0527 will be reduced once the effort increase of DOD W81XWH-21-1-0634 approved.

2P50CA186784 (Ellis) 08/01/20 – 07/31/25 3.0 CM (25%)

NIH/NCI

Project Title: Translational Research in Breast Cancer

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

Project 1: Basic Co-Leader – 1.8 CM (15%)

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

Core C Admin: Core Lead – 1.2 CM (10%)

Major Goals: The overall goal of the Administrative Core is to consolidate common support and administrative functions for improved efficiency, to assure quality control in record-keeping, services, and compliance issues, and to support the Director and Executive Committee in maintaining integration and communication among the components and individual investigators involved in the SPORE effort.

W81XWH-21-1-0106-***This grant*** 03/15/21-03/14/24 4.2CM (35%)

Department of Defense
Project Title: Therapeutic Targeting of Nuclear Hormone Receptors in Neurofibromin/NF1-Depleted Breast Cancer
Role: Initiating PI
Major Goals: The overall objective of this project is to assess what therapeutic opportunities are associated with neurofibromin loss in patients.
Specific Aims

1. We will perform molecular biology experiments to determine whether neurofibromin-loss causes AR activation in both ER+ and ER– breast cancer cells.
2. We will determine whether ER+ NF1– breast cancer is more sensitive to an AR-activating agent, or 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.

W81XWH-21-1-0634 ***New*** 08/01/21 – 07/31/23 2.4 CM (20%)
Department of Defense (Ellis)
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

RP180844 (Chang)-***Closed*** 08/31/18 - 08/31/21(NCE) 1.20 CM(10%)
CPRIT – HI/HR
Project Title: Regulating androgen receptor as a co-repressor by neurofibromin (NF1)
Grant Contact: Patty Moore - pmoore@cprit.texas.gov
Role: Principal Investigator
Major Goals: This project will investigate the hypothesis that NF1 is a co-repressor for AR as well as ER, so that its loss may increase AR (as well as ER) transcriptional activities in breast cancer cells
Specific Aims
1. We will assess whether NF1 functionally acts as an AR co-repressor by measuring AR transcriptional activity after *NF1*-silencing *in vitro*
2. We will assess the roles of anti-AR agents in treating NF1-deficient breast cancer by determining whether its growth can be pharmacologically blocked *in vitro*, as well as *in vivo* using xenograft mouse models

W81WH-16-1-0538 (Chang) 09/30/16-09/29/20(NCE) 3.24 CM(27%)
Department of Defense
Project Title: Direct Regulation of Estrogen Receptor Transcriptional Activity by NF1
Grants Contact: Jamie A. Shortall – Jamie.a.shortall.civ@mail.mil
Role: Initiating PI
Major Goals: The key objectives of this proposal are to define NF1's role in the control of ER's transcriptional activity and to assess the clinical significance of this finding by focusing on designing treatment strategies
Specific Aims
1. To define how NF1 regulates expression of ER target genes by investigating a direct interaction between NF1 and canonical ER transcriptional co-regulators
2. To establish a strategy to treat NF1-deficient ER^+ breast cancers by rationally combining anti-Ras and anti-ER approaches.

1R21CA226567-02 (Chang)-***Closed*** 06/04/18 - 05/31/21(NCE) 1.8 CM(15%)
NIH
Project Title: A Novel N-Ras Pathway DCIS to Basal-like Breast Cancer
Grant Contact: Viviana Knowles – Viviana.knowles@nih.gov
Role: Principal Investigator

Major Goals: Our overall research goal is to show that BLBC evolves from luminal cells during DCIS, driven by N-Ras.
Specific Aims
1. To assess whether BLBCs indeed evolve from luminal breast cancers, and whether this is driven by NRas in DCIS, studying both previously established DCIS cell lines and *patient-derived* primary DCIS cells both *in vitro* and *in vivo* using MIND xenografts
2. To assess whether formation of BLBC can be blocked early during DCIS by inhibiting the JAK2-STAT5-IL8 pathway downstream of N-Ras, using small molecule inhibitors in xenograft models

(Chang) 3/1/2016 – 2/28/2017 2.4CM(20%)
Willa and Ella Owen Medical Research Foundation
Project Title: Target Ras for Destruction as a Novel Treatment for Cancer
Grant Contact: Nancy Davis, Davis, ndavis@bcm.edu, 713-798-6194.
Role: Principal Investigator
Major Goals: This project is to assess the possibility that the stability of Ras proteins can be targeted by small molecule compounds that can later be developed into therapeutic agents.
Specific Aims
1. To use various mouse models to test whether flunarizine treatment can inhibit the growth and/or metastasis of basal-like breast cancer overexpressing N-Ras.
2. To define the mechanism by which flunarizine controls Ras degradation, by analyzing the interaction between Ras and key components involved in selective autophagy

1P50CA186784 (Osborne-PI; Chang-Project Leader) 3/1/15-8/31/16 0.12CM(1%)
NIH
Project Title: Target N-Ras for treating basal-like breast cancer- SPORE Developmental Project
Grant Contact: Viviana Knowles, viviana.knowles@nih.gov;
Role: Pilot project leader
Major Goals: The objective of this project is to seek means to target N-Ras in order to ultimately treat Basal-like Breast Cancer.
Specific Aims
1. To directly test whether FLN can be used to treat BLBC in vivo using mouse models
2. To fully investigate the mechanism that induces N-Ras degradation by examining the role of autophagy

SAC140059 Komen Leadership Grant (Ellis) 06/23/15-06/22/16 3CM(25%)
Susan G. Komen for the Cure
Project Title: Mechanisms of Endocrine Resistance in Estrogen Receptor Positive Breast Cancer
Grant contact: Jerome Jourquin, JJourquin@komen.org
Role: Co-Investigator
Major Goals: This project is to target aberrant cell survival mechanisms in ER+ breast cancer that permit late relapse by using genome matched pharmacological approaches, causing ER+ tumors to permanently regress.
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.

RP130135 (Chang) 06/01/13/-11/30/15 1.2 CM(10%)
Cancer Prevention and Research Institute of Texas
Project Title: Comprehensive identification of all human Ras effectors to define mechanisms of Ras-induced malignancy and potential drug targets.

Grant Contact: Michael Brown, mbrown@cprit.state.tx.us
Role: Principal Investigator
Major Goals: The major goal is to use a new technology to isolate all human Ras effectors and test them for relevance to tumor formation.

Specific Aims
1. To construct new Gateway compatible libraries from the ORFeome collection to ultimately cover the whole human genome.
2. To screen for new Ras effectors using N-Ras and K-Ras-4B as baits in live human cells.
3. To functionally validate the isolated Ras effectors for their roles in tumorigenesis.

Overlap:
None