

AWARD NUMBER: W81XWH-18-1-0034

TITLE: Mismatch Repair Loss Renders ER+/HER2- Breast Cancer Susceptible to HER2/3 Inhibition

PRINCIPAL INVESTIGATOR: Svasti Haricharan

CONTRACTING ORGANIZATION: Sanford Burnham Prebys Medical Discovery Institute  
La Jolla, CA 92037

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# REPORT DOCUMENTATION PAGE

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> The objective of this proposed study is to investigate a role for HER2/3 activation in MutLdefective ER+ breast cancer progression and resistance to endocrine therapy. By targeting HER2/3 signaling and key nodes of adaptive kinome response, we aim to significantly improve patient disease-specific survival.					
<b>15. SUBJECT TERMS</b> HER2 inhibitors, endocrine treatment resistance, growth factor signaling, ER+ breast cancer, DNA damage repair, mismatch repair					
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## **1. INTRODUCTION**

More than 70% of breast cancer is estrogen receptor positive (ER+) and is treated with endocrine therapy, which targets the ER-pathway. While the majority of patients respond to treatment, ~30% of patients are resistant. This resistant subset is a significant contributor to the >40,000 breast cancer-related deaths that occur every year in the US. Activation of HER signaling has been previously suggested to induce endocrine therapy resistance. The HER family of tyrosine kinase receptors consists of EGFR, HER2, HER3 and HER4, and they are all known oncogenes and growth promoters. However, clinical trials incorporating drugs targeting EGFR/HER2 and/or downstream signaling pathways (PI3K/AKT/mTOR) into endocrine treatment regimens have obtained mixed results. This failure is potentially explained by a lack of predictive biomarkers to demarcate patients most likely to benefit from such targeted therapies.

Recently, we identified that loss of mismatch repair (MMR), specifically of the MutL complex consisting of MLH1, PMS1 and PMS2 genes, causes endocrine therapy resistance in ER+ breast cancer cells. To identify more efficacious, preferably cytotoxic therapeutic targets in MutL-defective ER+ breast tumors, we performed a proteomics screen on MCF7 cells stably engineered to downregulate MLH1, PMS1 or PMS2 (shMLH1, shPMS1, shPMS2) collectively termed shMutL cells. The proteomic response of these cells to the endocrine therapy, fulvestrant (an ER degrader), differed from that of control (shLuc) MCF7 cells in one important, druggable way: shMutL cells upregulated HER2/3 signaling. This finding is completely novel and presents a unique opportunity to exploit existing HER inhibitors to successfully treat endocrine therapy resistant ER+ breast cancer patients using rational drug combinations.

While HER2/3 inhibitors have been recommended for endocrine therapy resistant ER+ breast tumors in the past, clinical trials suggest that only an undefined subset of patients respond to this treatment, indicating a critical need for stratification based on predictive biomarkers. In the proposed study, we will investigate a role for MutL in predicting response to HER2/3 inhibitors in up to 30% of endocrine therapy resistant ER+/HER2- breast cancer.

## **2. KEYWORDS**

HER2 inhibitors, endocrine treatment resistance, growth factor signaling, ER+ breast cancer, DNA damage repair, mismatch repair

## **3. ACCOMPLISHMENTS**

### ***What were the major goals of the project?***

Major goals of the project were to (a) Validate activation of HER2/3 signaling in MutL-defective ER+/HER2- breast cancer cells (b) Investigate HER2/3 activation and signaling mechanisms in MutL-deficient ER+ breast cancer (c) Test efficacy of HER inhibition in decreasing MutL-defective ER+ breast cancer growth on endocrine treatment.

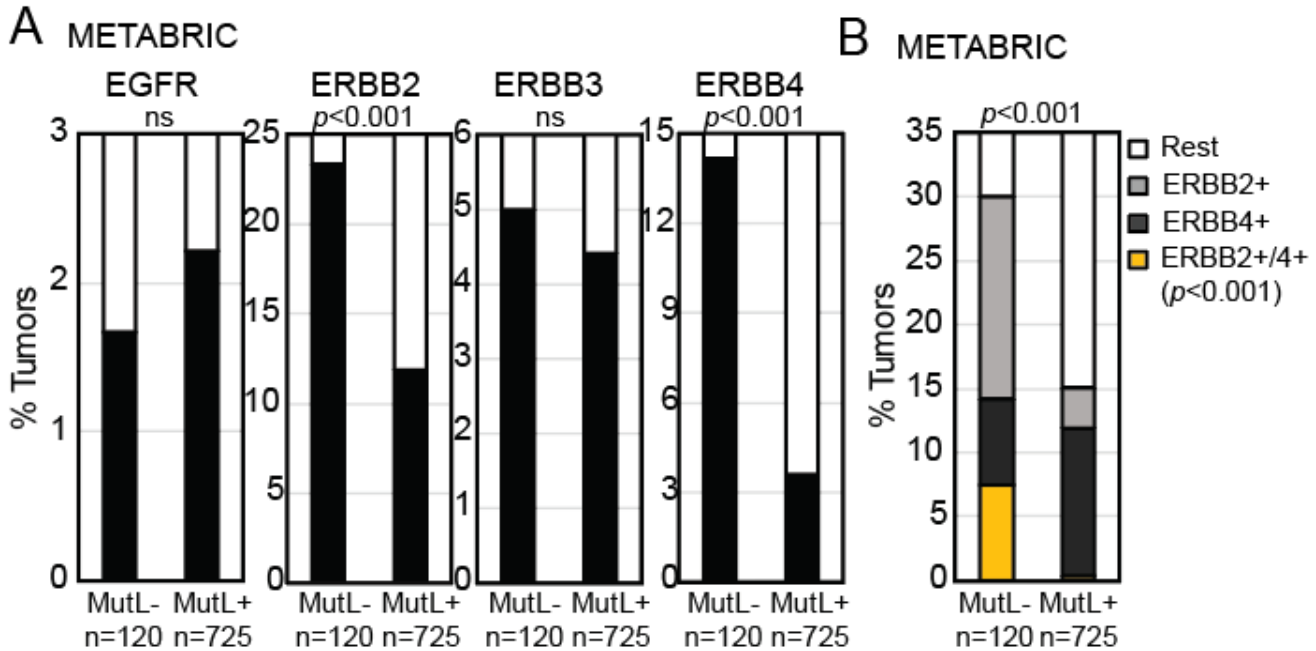
### ***What was accomplished under these goals?***

In the first year, Major Task 1 as outlined in the SOW was completed. Informatics analysis of patient tumors outlined in task 1.1 was conducted by Dr. Haricharan, surprisingly revealing that HER4, rather than HER3 is upregulated along with HER2 in ER+ patient tumors that are defective in mismatch repair (Fig 1). This finding was also confirmed in TCGA dataset (Fig 2). Accordingly, we pursued investigations into HER2 and HER4 co-upregulation in experimental model systems thereafter, rather than HER3 as initially outlined. As specified in task 1.2, IF (Fig 3) experiments were conducted in tumor sections from previously generated WHIM20 PDX line after

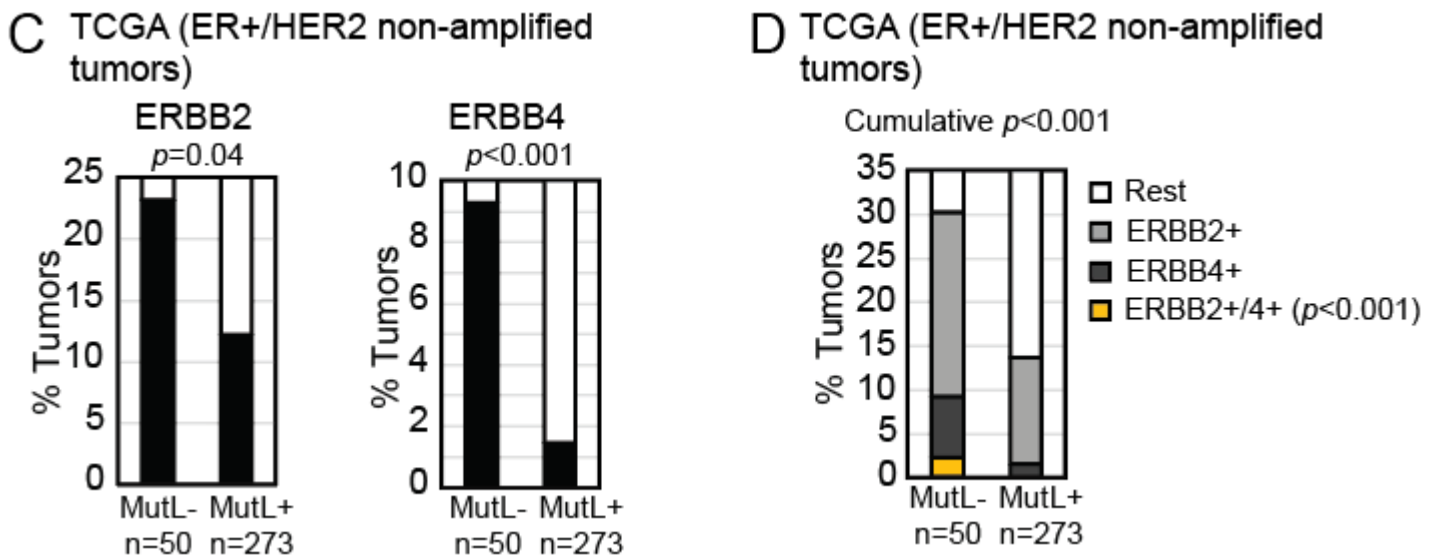
fulvestrant treatment to optimize antibodies against HER2 and HER4. HER3 was not assessed since there was no clinical correlations identified. Next as per task 1.3, IF was conducted after fulvestrant treatment in vitro (Fig 4). These data indicated significant co-upregulation of active pHer2 and HER4 after fulvestrant treatment in MutL-defective ER+ breast cancer cells. Confirmation in xenograft tumor sections is on-going. The final task completed, as per the SOW was 2.1. Western blotting was conducted and confirmed upregulation of both pHER2 and pHER4 specifically after fulvestrant treatment (Fig 5). Probing for EGFR and HER3 will be completed in the next two months once antibody optimization is completed.

**Major Task 1 Subtask 1**

(Fig 1) METABRIC data analysis: upregulation of ERBB2 (HER2) and ERBB4 (HER4) is significantly enriched in MutL- ER+ tumors as assayed by gene expression and co-upregulation is also observed in this subset.



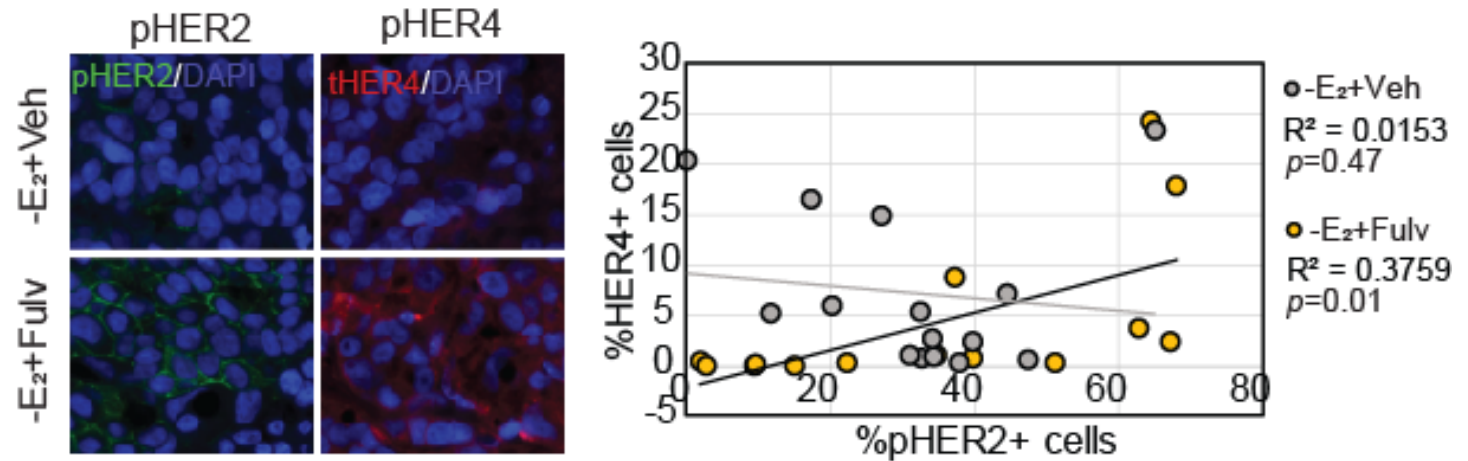
(Fig 2) TCGA data analysis: enrichment for upregulation of ERBB2 (HER2) and ERBB4 (HER4) in MutL- ER+ tumors is confirmed in TCGA. These data prompted us to begin investigating the role of HER2 and HER4, rather than HER3, in inducing endocrine treatment resistant growth in MutL- cells.



## Major Task 1 Subtask 2

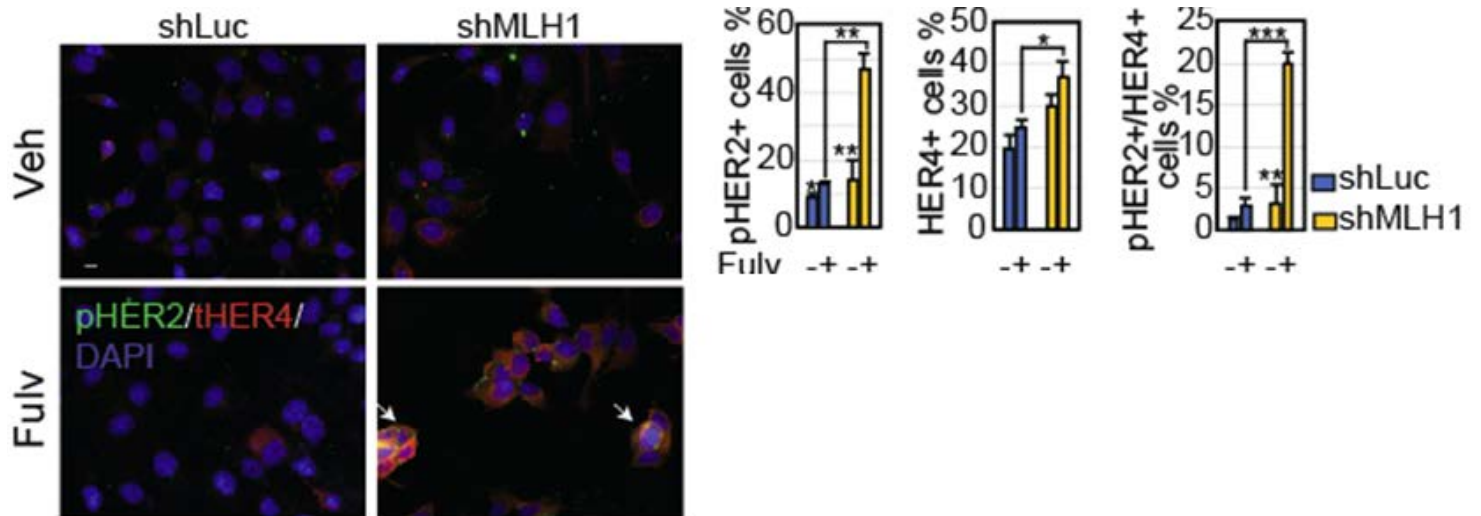
(Fig 3) Immunocytochemistry optimization: WHIM20 tumors demonstrating co-upregulation of HER2 and HER4 at the cytoplasm/membrane upon fulvestrant treatment. Experiment was repeated across more than 10 PDX tumors. Representative images shown below.

### E WHIM20 (PMS2 mutant)



## Major Task 1 Subtask 3

(Fig 4) Immunofluorescence: MCF7 shMLH1 cells confirm colocalization of HER2 and HER4 at the membrane in vitro after treatment with fulvestrant, unlike shLuc cells. Experiments were repeated three times. Representative images shown below.



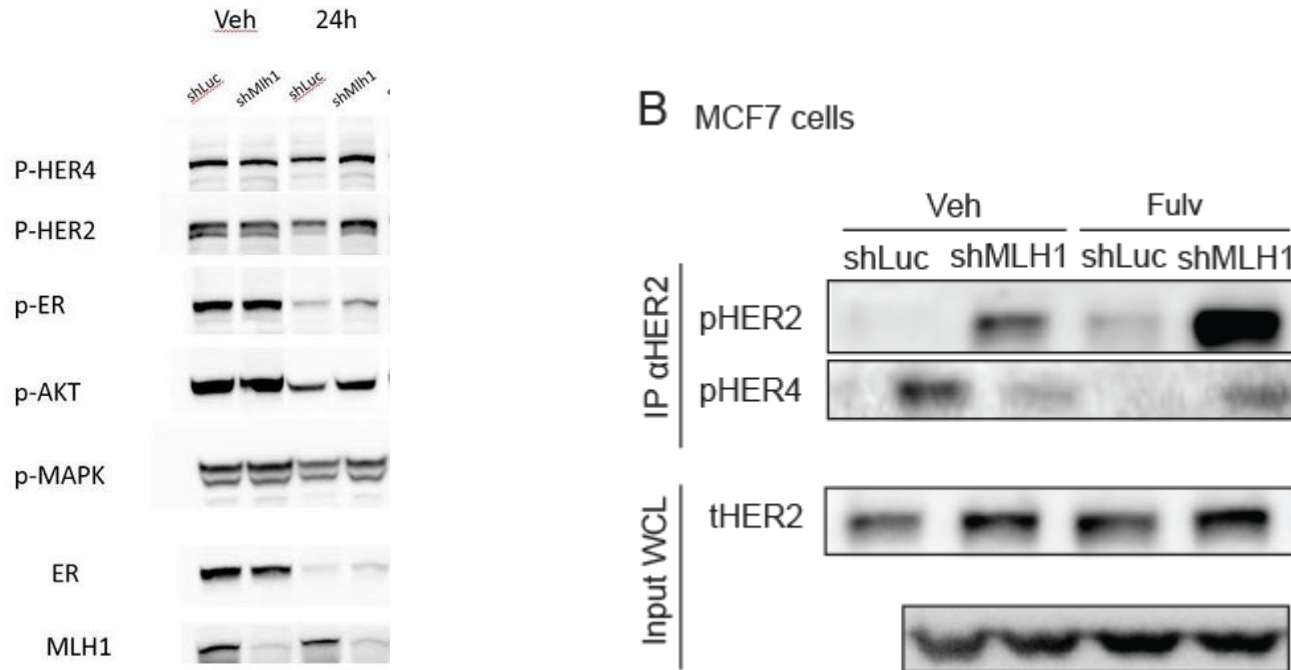
## Major Task 1 Subtask 5

ACURO approval for animal experiments was received.

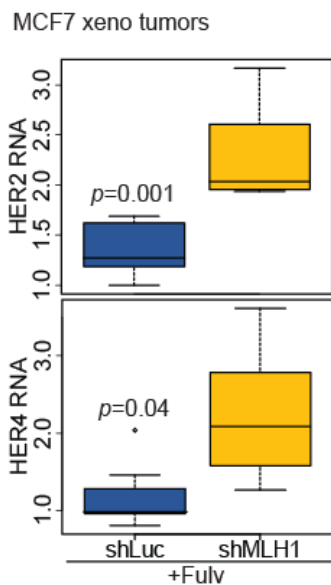
## Major Task 2 Subtask 1

This subtask confronted us with a challenge since it became quickly evident that antibodies would have to be optimized and a time course would have to be performed to identify the optimal conditions for the experiment. Optimization and troubleshooting delayed the completion of the aim. However, the critical experiment in MCF7 cells has been completed, as presented below.

(Fig 5) Western blotting: MCF7 shMLH1 cells demonstrate upregulation of both pHER4 and pHER2 in response to fulvestrant within 24 hours of treatment, relative to shLuc cells (left). Additionally, IP for HER2 in MCF7 shLuc and shMLH1 cells treated with fulvestrant shows increased active HER2 in shMLH1 cells (right). These data are in process of being confirmed in T47D cells, and in lysates from MCF7 shLuc and shMLH1 xenograft tumors and HCI-005 PDX tumors.



(Fig 6) qRT-PCR: MCF7 shMLH1 xenograft tumors demonstrate upregulation of both HER2 and HER4 mRNA in response to fulvestrant, relative to shLuc cells.



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

Results were presented by Dr. Haricharan at the Gordon Research Conference, which aided in networking and collaborations for her lab, as described below.

***How were the results disseminated to communities of interest?***

Preliminary results from the project, establishing the role of DNA damage repair defects in inducing endocrine treatment resistance, were presented at the Gordon Research Conference. The submitted abstract was selected for a short poster talk. Presentation of this work allowed stimulating discussion and feedback from the scientific community regarding the progress of the study and the novel role for DNA damage repair defects in ER+ breast cancer treatment response, the basic premise of the work proposed for this award.

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

During the next reporting period, IHC for HER2 and HER4 in patient tumors from P024 will be completed, and Drs. Haricharan and Kavuri will also work together to submit a manuscript describing the frequency and incidence of HER2/HER4 upregulation in MutL- ER+ patient tumors. Additionally, Western blotting for HER2 and HER4 will be validated in T47D cells, xenograft tumor and PDX paraffin-embedded sections, in completion of goal 1. Additionally, preliminary data regarding upregulation of growth factor secretion in MutL- ER+ breast cancer cells will be validated in multiple cell lines using ELISA and in PDX tumors. Effect of these growth factors on activation of HER2 and HER4 upon fulvestrant treatment will be functionally validated in 2D and 3D culture.

**4. IMPACT**

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

Nothing to Report.

***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**

***Changes in approach and reasons for change.***

The initial proposal sought to understand the role of HER2/HER3 co-upregulation in MutL- ER+ breast cancer cells. However, additional informatics and functional experiments indicated that HER4 is the partner for HER2 in mediating endocrine treatment resistant growth. Therefore, the rest of the study will be completed as specified except only that HER4 will be substitute for HER3 in each experiment.



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

This award was transferred from Baylor College of Medicine as of 08/31/2018 and initiated at Sanford Burnham Prebys Medical Discovery Institute 01/15/2019. The project period has been extended to 07/14/2021 due to the transfer lapse.

*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.*

Poster presentation at Gordon Research Conference.

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

Nothing to Report.

*Technologies or techniques.*

Nothing to Report.

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

Nothing to Report.

*Other products.*

Nothing to Report.

## **7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

*What individuals have worked on the project?*

The below information represents the participant efforts for the awarded period at SBP of 01/15/2019. There were no changes to personnel roles/contributions.

Haricharan, Svasti, Principal Investigator – 0.25 person months

Punturi, Nindo, Research Assistant – 4.5 person months

Mazumder, Aloran, Postdoctoral Associate – 0.45 person months

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

Yes, other support attached.

*What other organizations were involved as partners?*

Baylor College of Medicine, Dr. Kavuri, Partnering PI

**8. SPECIAL REPORTING REQUIREMENTS**

Nothing to report.

**9. APPENDICES**

Nothing to report.

## CHANGES IN ACTIVE SUPPORT

### HARICHARAN, SVASTI

#### ACTIVE

K22 CA229613-01 (Haricharan) 09/01/2018 – 08/31/2021 9 CM (75%)  
NIH/NCI \$408,804 total direct costs

A Pan-Cancer Role for MutL Loss in Inducing Treatment Resistance

The goal of this project is two-fold:

- a) to establish a multi-cancer role for MutL loss in inducing poor clinical outcome and
- b) to uncover therapeutic strategies that can be repurposed to target MutL<sup>-</sup> breast, bladder and colorectal cancer.

Specific Aims:

- 1) Investigate functional impact of MutL dysregulation in ER<sup>+</sup> breast, bladder and colorectal cancer.
  - 1a) Validate effect of MutL vs MutS inhibition on response to standard-of-care in bladder and colorectal cancer.
  - 1b) Test impact of MLH1 mutations identified in primary human tumors on response to current standard- of-care.
- 2) Identify alternative targeted treatments for MutL- ER<sup>+</sup> breast, bladder and colorectal cancer.
  - 2a) Validate a role for MutL-loss in inducing sensitivity to CDK4/6 inhibition in bladder and colorectal cancer.
  - 2b) Investigate the efficacy of a combination of CDK4/6 and Bcl inhibitors in inhibiting growth of MutL- ER<sup>+</sup> breast, bladder and colorectal tumors.

Grants Management Specialist: Nailah Agyemann, (240) 276-6290, agyemann@mail.nih.gov

Role: PI

Overlap: No scientific or budgetary overlap.

(NEW)

U54 CA233223 (Mitsiades) 09/20/2018 – 06/30/2023 0.3 CM (2.5%)  
Baylor College of Medicine/NIH \$20,100 total direct costs

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 (Project 2: Targeting Estrogen Receptor and DNA Damage Repair Disparities in African American and Hispanic/Latino Breast Cancer Using Patient-Derived Breast Cancer Xenografts)

Dr. Haricharan will work with Dr. Ellis' group to oversee design and implementation of PDX tumor growth and drugging experiments, and provide expertise in intraductal mammary gland injections. Additionally, she will oversee downstream analyses including sequencing and RNA seq, and work with Drs. Ellis and Kim to identify druggable targets from incorporated proteogenomics data.

Specific Aims (Haricharan subaward):

- 1) Analyze alterations (mutations and fusions) in ESR1 and downstream signaling effectors and correlate their incidence with endocrine treatment response.
- 2) Synthesize genomic signatures of DNA repair defects with alterations to specific DNA damage repair genes, and associate these with response to endocrine treatment, PARP inhibitors and CDK4/6 inhibitors.
- 3) Contribute experience and expertise to weighing the combined geno-proteomic data across PDX lines from ethnic groups, with the network analysis to identify candidate therapeutic alternatives to further investigate.

Grants Management Specialist: Ashley Salo, 240-276-5656, Ashley.salo@nih.gov

Role: Subaward PI

Overlap: No scientific or budgetary overlap.

(NEW)

CCR18548157 (Haricharan)

10/23/2018 – 10/22/2021

1.2 CM (10%)

Susan G. Komen

\$299,967 total direct costs

Mismatch Repair Defects and Endocrine Therapy Resistance in ER+ Breast Cancer

We will test the hypothesis that MutL-deficiency can stratify estrogen receptor positive (ER<sup>+</sup>) breast cancer patients for FDA-approved, non-endocrine therapies. There is no overlap with other funded grants.

Specific Aims:

- 1) Detect MutL loss in ER+ breast cancer. Here, we will test the feasibility of adapting existing immunohistochemical (IHC) assays for MutL proteins to ER+ breast cancer using MutL- cell lines and patient-derived xenograft (PDX) tumors.
- 2) Test efficacy of MutL loss as a stratifier for Bcl inhibitors in ER+ breast cancer patients. In this aim, we will test whether MutL- ER+ breast cancer cells and PDXs are preferentially sensitive to Bcl inhibition.
- 3) Discover novel therapeutic targets in endocrine therapy resistant, MutL- ER+ breast cancer. We will use mass spectrometric analysis of the entire proteomes of ~40 ER+ PDXs to identify novel druggable targets in endocrine therapy resistant ER cancer.

Research Grants Manager: David Vaught, 972-701-2039, [dvaught@komen.org](mailto:dvaught@komen.org)

Role: PI

Overlap: No scientific or budgetary overlap.