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

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14. ABSTRACT

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.

15. SUBJECT TERMS
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?

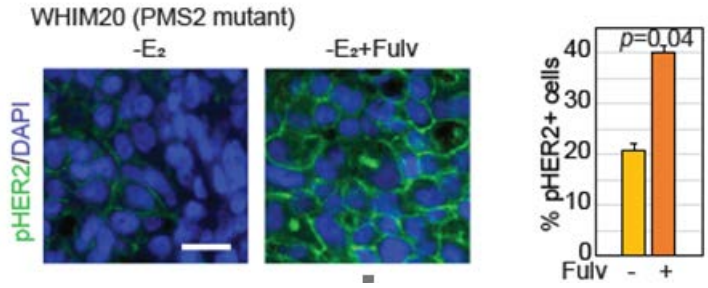
Major goals of the project were to (a) Validate activation of HER2/3 signaling in MutL-defective ER+/HER2- breast cancer cells (b) Investigate HER2/3 activation and signaling mechanisms in MutL-deficient ER+ breast cancer (c) Test efficacy of HER inhibition in decreasing MutL-defective ER+ breast cancer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

What was accomplished under these goals?

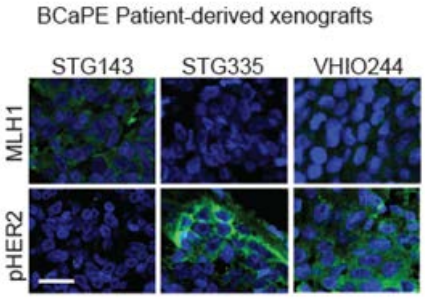
In year 2, milestone#1 was achieved in that a co-corresponding manuscript describing activation of HER2 in MutL- ER+ breast cancer cells was submitted and is under review at *Science Translational Medicine.*
In terms of experiments, major task 2 subtask 2 (Fig 1) was completed and HER2 upregulation was confirmed in MutL- PDX line, WHIM20 after treatment with fulvestrant. In addition, two more MutL- PDX lines were identified and IF of these lines for HER2 and MLH1 were conducted (Fig 2) to further support our hypothesis. As outlined in the statement of work, major task 3 subtask 1 (Fig 3, 5-6) was also completed and we identified BTC as one ligand that is upregulated in response to fulvestrant in shMLH1 ER+ breast cancer cell lines and xenograft tumors. These data were validated by analysis of patient tumors as well (Fig 4). Finally, major task 3 subtask 2 was completed and upregulation of HER2 in response to BTC was confirmed by immunofluorescence (Fig 7) and Western blotting (Fig 8). ELISA experiments are underway to complete proteomic assessment of ligand secretion in MutL- cells but had to be halted in response to the COVID-19 outbreak resulting in closure of SBP.

Major Task 2 Subtask 2

(Fig 1) IF for pHER2 in MutL- ER+ PDX line, WHIM20 after addition of Fulvestrant.

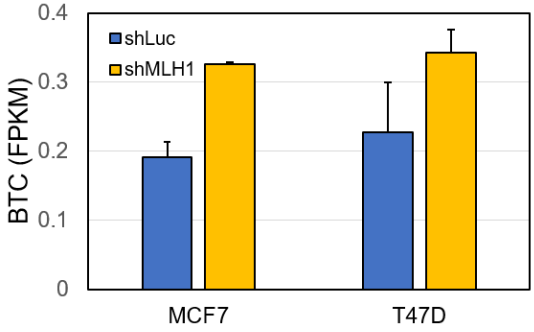


(Fig 2) IF for pHER2 in two additional MutL- ER+ PDX lines (including one MutL+ ER+ PDX line as control) from the BCaPE initiative at CRUK.

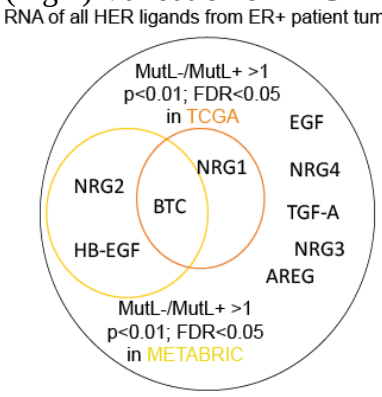


Major Task 3 Subtask 1

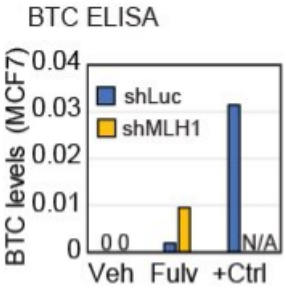
(Fig 3) RNAseq data for BTC in MCF7 (p=0.04) and T47D (p=0.03) shLuc and shMLH1 ER+ breast cancer cell lines after treatment with fulvestrant.



(Fig 4) Validation of BTC RNA upregulation in MutL- ER+ patient tumors from two independent datasets.

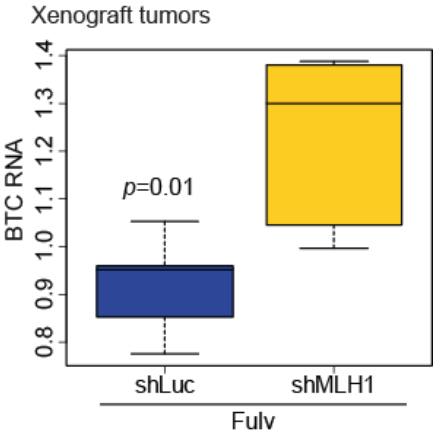


(Fig 5) ELISA for BTC in MCF7 shLuc and shMLH1 ER+ breast cancer cell lines.



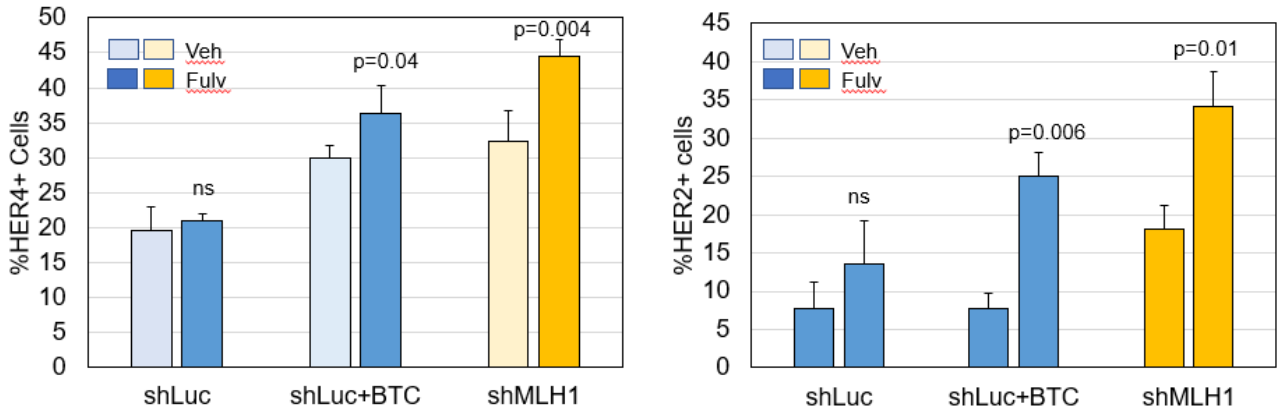
In progress: Confirmation in T47D cells

(Fig 6) qRT-PCR data validating upregulation of BTC RNA levels in MCF7 shMLH1 xenograft tumors after fulvestrant treatment.



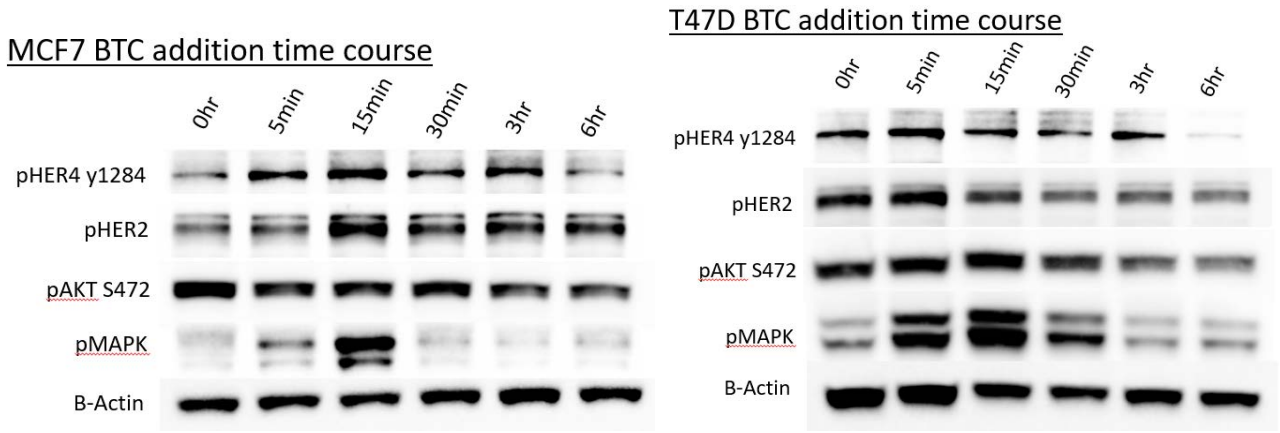
Major Task 3 Subtask 2

(Fig 7) Upregulation of HER2 in response to BTC by immunofluorescence in MCF7 cells.



In progress: Confirmation in T47D cells

(Fig 8) Upregulation of HER2 in response to BTC by Western blotting.



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

Several new collaborations have been started based on the results of this project. The first collaboration is with Drs. Nicola Fusco and Konstantinos Venetis at the IRCCS European Institute of Oncology. This collaboration has also resulted in a collaborative editorial in progress. Secondly, Dr. Haricharan has initiated a collaboration with Dr. Rebecca Shatsky at UCSD to test MLH1 and HER2 association in metastatic ER+ breast cancer patient tumor samples. They are currently applying for funding to get this project off the ground. Finally, Dr. Haricharan has initiated a collaboration with Dr. Stephen Shiao at Cedar Sinai in LA. Together, they plan to conduct a preliminary Phase II clinical trial to test efficacy of HER2 inhibition in combination with endocrine therapy in MutL-ER+/HER2- breast cancer patients. They are applying for an internal grant to provide preliminary supportive data for this project, as well as Department of Defense Breakthrough Level 3 grant to support further studies and the proposed clinical trial. Dr. Haricharan also served as a reviewer for the DoD Breakthrough awards in 2019.

The Sanford Burnham Prebys Medical Discovery Institute (SBP) Office of Education, Training & International Services (OETIS) oversees and coordinates an annual individual development planning (IDP) process for all postdocs at the Institute. The focus of the IDP process at SBP is the career goal of the postdoc; identification of what skills, knowledge, and accomplishments will be necessary for the postdoc to obtain a desired independent position following training; and identification of training and professional development opportunities that are available for the postdoc to obtain the necessary skills and knowledge. The SBP Office of Education, Training & International Services provides guidance and advising to both postdocs and PIs throughout the postdoc's training with respect to developing IDPs and preparing for a successful transition to independence post-training. The SBP Office of Education, Training & International Services also maintains webpages containing comprehensive resources on career path identification, career planning, and creating an IDP that can be utilized in conjunction with the formal annual IDP process.

The SBP IDP process includes two components:

I) <u>First-Year IDP (effective in 2014)</u>. Within the first 3 months of beginning postdoctoral training at SBP, all postdocs receive and fill out an initial "planning and expectations" document to discuss with their PI. This document serves as the foundation for their postdoctoral IDP and is designed to facilitate discussion between the PI and new postdoc regarding goals and expectations for the first year of training, as well as stimulate initial discussions about long-term career goals and training plans.

II) <u>Postdoctoral IDP (effective January 2013)</u>. At the end of the first year of training SBP postdocs receive notification that it is time to update their IDP, and they receive the information they included in their first-year planning and expectations document in the form of a full IDP that they can update with their accomplishments over the past year and their goals for the coming year, mid-term future, and long-term future. Each subsequent year of their postdoctoral training, postdocs will receive notification and the previous year's IDP form to update

and expand. The IDP forms are designed to build upon each previous year as well as provide a solid foundation from which a postdoc can easily build his or her CV/resume.

Dr. Haricharan and Dr. Mazumder participated in Part II of the IDP Process this past year.

How were the results disseminated to communities of interest?

In the past year, Dr. Haricharan has identified a patient advocate, Karen McDonald who partners closely with her lab in disseminating results to communities of interest. Further, Dr. Haricharan was an invited speaker at the Fleet Science Lecture series at the San Diego Museum of Science, and the Komen Race for the Cure Inauguration ceremony where she presented some of her work to the general public. Further, Dr. Haricharan and her lab participated in an interview for ABC describing recent breakthroughs in breast cancer research (https://abc30.com/5347545/) and in the Komen Race for the Cure Public Service Announcement aired on CBS (https://www.youtube.com/watch?v=p2AzReqsmB8&feature=youtu.be).

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

During the next reporting period, functional experiments with conditioned media and testing sufficiency and necessity of identified ligands will be conducted. These will constitute a second manuscript for submission. Additionally, IHC for MLH1 and HER2 in patient tumor samples will be completed with Dr. Kavuri at BCM and Dr. Shatsky at UCSD will be put together as a third manuscript.

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.

None.

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

The IHC for pHER2 in patient samples to be done at BCM is still under way. Pilot experiments have been successfully completed and antibodies provided to the BCM Histology core. A new automated microscopy

instrument has been instituted in the BCM Histology core and protocols are under optimization. Once completed, IHC for pHER2 and MLH1 in patient samples should be completed in a matter of weeks. There has been a brief halt due to the COVID-19 outbreak, which has resulted in BCM and SBP closure. But once business is resumed these experiments will be completed with no anticipated hurdles.

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.

Publications: We have submitted a manuscript describing our findings, which is currently under review: Punturi, N.B., Seker, S., Devarakonda, V., Kalra, R. Chen, C., Mazumder, A., Li, S., Primeau, T., Ellis, M.J., Kavuri, S.M., Haricharan, H. (2020) "Mismatch repair deficiency predicts response to HER2 blockade in HER2-negative breast cancer". Under review at *Science Translational Medicine*

Presentations: Abstract accepted for poster presentation at AACR 2020

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?

Haricharan, Svasti (Principal Investigator) – 0.91 person months (*The total 0.91 calendar months' funding support was paid entirely by the grant and not supplemented from any discretionary or other funding source*).

Punturi, Nindo (Research Assistant) – 12 person months

Mazumder, Aloran (Postdoctoral Associate) – 1.8 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?

Nothing to Report.

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.