AD_____

AWARD NUMBER: W81XWH-15-1-0045

TITLE:
Defining High-Risk Precursor Signaling to Advance Breast Cancer Risk Assessment and Prevention

PRINCIPAL INVESTIGATOR: Dr. Leif Ellisen

CONTRACTING ORGANIZATION: Massachusetts General Hospital Boston, MA 02114-2696

REPORT DATE: May 2018

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
May 2018	Final	1 Mar 2015 - 28 Feb 2018

4. TITLE AND SUBTITLE

Defining High-Risk Precursor Signaling to Advance Breast Cancer Risk Assessment and Prevention

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0045

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
DR. LEIF ELLISEN

E-Mail: LELLISEN@MGH.HARVARD.EDU

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

MASSACHUSETTS GENERAL
HOSPITAL
55 FRUIT STREET
BOSTON, MA 02114-2621

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Making a major impact on the incidence and lethality of breast cancer will require a detailed understanding of the earliest tissue changes that ultimately drive the process of breast cancer development. There is no substitute for the ability to define and understand the early, pre-malignant changes as they occur in women who are breast cancer-predisposed. One group of women at high breast cancer risk (up to 80% lifetime breast cancer risk) are those who have inherited mutations in the BRCA1 and BRCA2 genes. Currently, the only way these women can eliminate their risk is to undergo bilateral mastectomy before developing cancer. We have established an IRB-approved protocol that allows us to collect and analyze a portion of this tissue. Here, we propose detailed functional and molecular analysis of these tissues in order to reveal critical early steps in breast cancer development. We will then test how reversing these changes can prevent breast cancer in well-established animal models. These studies are likely to lead directly to clinical trials of new approaches to prevent breast cancer.

15. SUBJECT TERMS
Breast cancer; BRCA1/2; cancer prevention; paracrine signaling

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
				24	USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	U		

Table of Contents

Page

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Making a major impact on breast cancer will require a detailed understanding of the earliest tissue changes. One group of women at high breast cancer risk are those with BRCA1 and BRCA2 mutations. Currently, the only way these women can eliminate their risk is to undergo bilateral mastectomy before developing cancer. Here, we propose detailed functional and molecular analysis of these tissues in order to reveal critical early steps in breast cancer development. We will then test how reversing these changes can prevent breast cancer in well-established animal models. These studies should lead directly to clinical trials of new approaches to prevent breast cancer.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Breast cancer; BRCA1/2; cancer prevention; paracrine signaling

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1: Functional analysis of progenitor and stem cells in high-risk tissues.

Major Task 1Functional quantitation (100% Completed in Months 1-24)
Major Task 2 Functional analysis (100% Completed in Months 1-12)
Major Task 3 Signaling Analysis (100% Completed in Months 1-12)

Aim 2: Discover and validate new pathways activated in cancer-predisposed tissues.

Major Task 1Transcriptomics (100% Completed in Months 1-24)
Major Task 2 Sub-clonal genomics (100% Completed in Months 1-24)
Major Task 3 Integrated bioinformatics (100% Completed in Months 1-24)

Aim 3: Block abnormal signaling in vitro and in vivo

Major Task 1Reverse abnormal signaling (50% Completed in Months 1-24)
Major Task 2 In vivo cancer models (In Process)

What was accomplished under these goals?

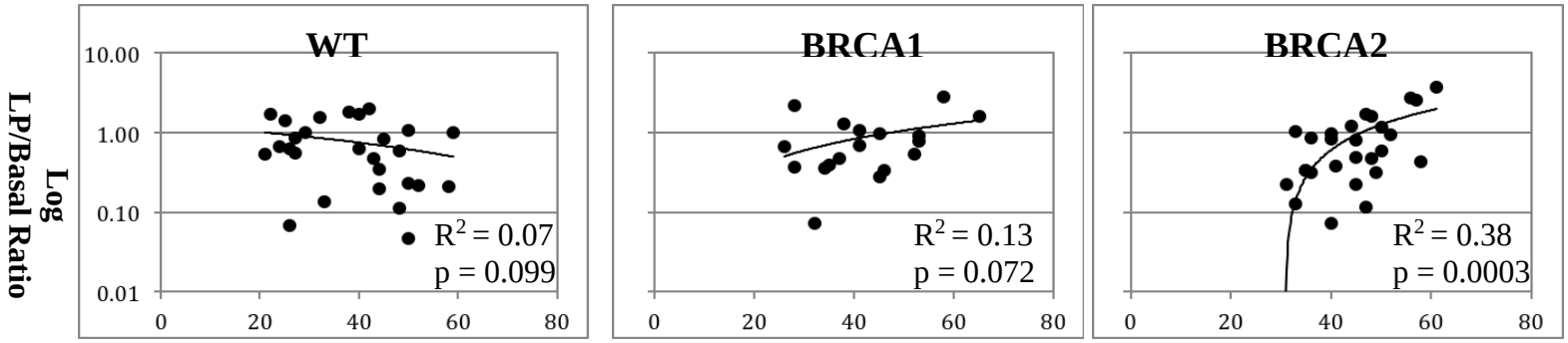
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

NB: We are pleased to share the remarkable accomplishments under this award to date. In order to facilitate review, accomplishments are now organized and enumerated based on tasks corresponding to the original SOW for this award. Accomplishments are supported by tables and figures to facilitate review.

Specific Aim 1: Functional analysis of progenitor and stem cells in high-risk tissues.

Major Task 1: Quantitation of LP (Luminal Progenitor) and basal stem cell (MASC) populations

A. Quantitation of LP and basal stem cell (MASC) populations

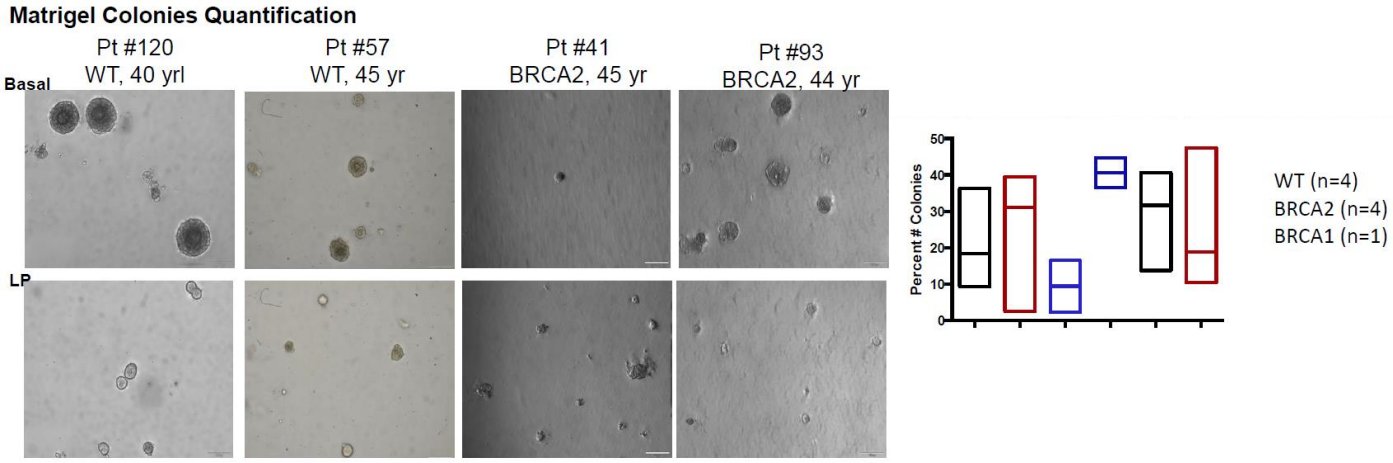


We have continued to add patients to the cohorts between months 12 and 24. (This reporting period). Latest data on all current cohorts are demonstrated above.
WT (n = 27)
BRCA1 (n = 18)
BRCA2 (n = 27)

Methods and accomplishments (Figure 1 above):
Method: Primary mammary tissues from each patient underwent enzymatic digestion, followed by specific antibody labeling and FACS as described in detail in the original proposal.

Accomplishments: Shown is the ratio of LP to basal (MASC) population for each patient. We accomplished the novel and remarkable finding that there is a near significant age-associated increase in ratio of LP/to basal cells specifically in BRCA2 carriers. This finding supports the hypothesis of a deregulation of the LP population in BRCA2 carriers.

B. CFU and mammosphere assays for functional quantitation
Methods and accomplishments: (Figure 2 below).
Method: Primary FACS-sorted mammary epithelial cells were plated in matrigel and percent of plated cells forming colonies was tabulated.

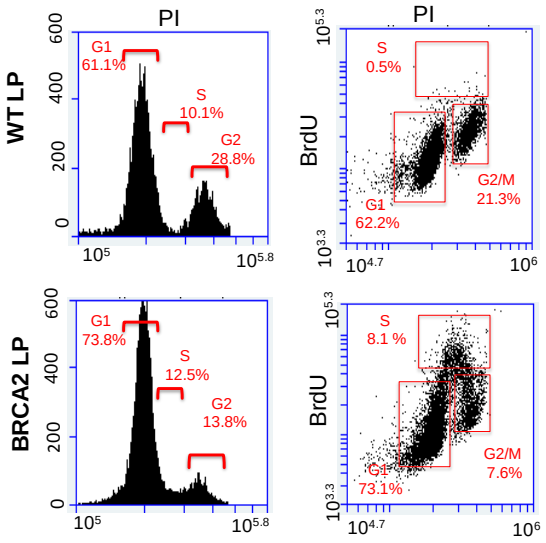


Accomplishments: Shown at left are representative matrigel assays: top, basal cells; bottom, LP cells. Shown at right is the summary: box shows range, and horizontal line shows mean; left boxes show basal cells, right boxes show LP cells; black: WT; blue: BRCA1; red: BRCA2. These results support the hypothesis of replication stress in BRCA2 LPs leading to more limited growth in matrigel.

<u>Major Task 2: Functional analysis of LP and basal stem cell (MASC) populations</u>

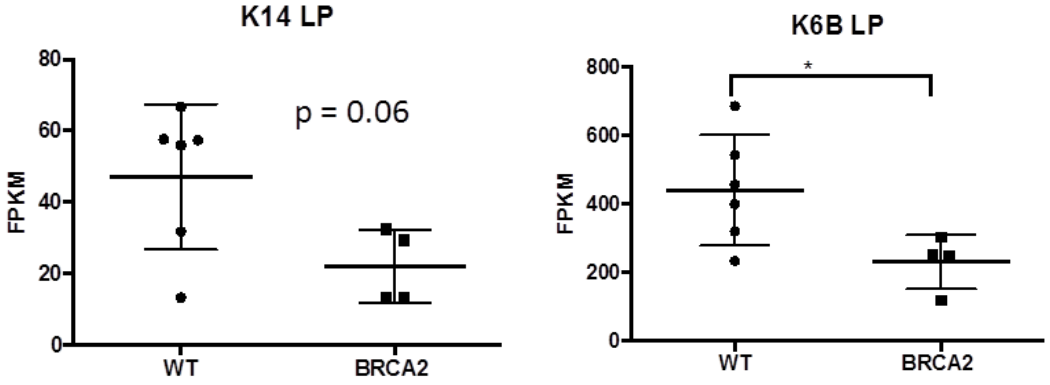
A. *CFU assays in presence and absence of growth factors.*
Methods and accomplishments: (Figure below).

Methods: Primary FACS sorted mammary epithelial LP cells from WT and BRCA2 carrier patients were stained with propidium iodide (PI) (left graphs) or PI and BrDU (right graphs) and quantitated by FACS. Y-axis at left = cell number; Y-axis at right =BrDU intensity. X-axis = PI intensity in both graphs.



Accomplishments: These studies demonstrate for the first time a deregulated proliferation of primary FACS-sorted LP cells from a BRCA2 carrier. We hypothesize that this deregulated proliferation is reflected in the deregulated LP cell number shown in Figure on page 5.

B. Assessment of bi-lineage differentiation
Methods and accomplishments: (Figure below).



Methods: RNA was extracted and sequenced from primary FACS-sorted mammary epithelia from non-carrier (WT) control patients and BRCA2 carriers. Genes related to cell fate and cell lineage were analyzed for alterations in the control versus BRCA2 populations. Graphs show gene expression (Y-axis, arbitrary values) of Keratin 14 (K14) and Keratin 6B (K6B), both basal differentiation markers, from non-carrier subjects (circles) and BRCA2 mutation carriers (squares). Each spot represents gene expression in one patient's LP sample.Vertical bars show standard deviation and middle horizontal bars show means. *P=0.06 for both comparisons.

Accomplishments: These experiments show for the first time decreased basal-like differentiation in LP cells from BRCA2 mutation carriers. These findings are consistent with the hypothesis that not only cell numbers but also cell fate is deregulated in the BRCA2 LP population.

<u>Major Task 3: Analyze signaling in high-risk tissues in vivo.</u>
IHC to analyze signaling in high risk tissues and controls in vivo
Methods and accomplishments: (Figures below).

Methods: Fixed tissues from control patients and BRCA1/2 carriers were subjected to immunohistochemistry for the proteins detailed below. Figure 5 below shows Ki67 (proliferation marker). Clinical characteristics of each subject are indicated. Fractions refer to number of KI67 positive epithelial cells/total number of cells counted.

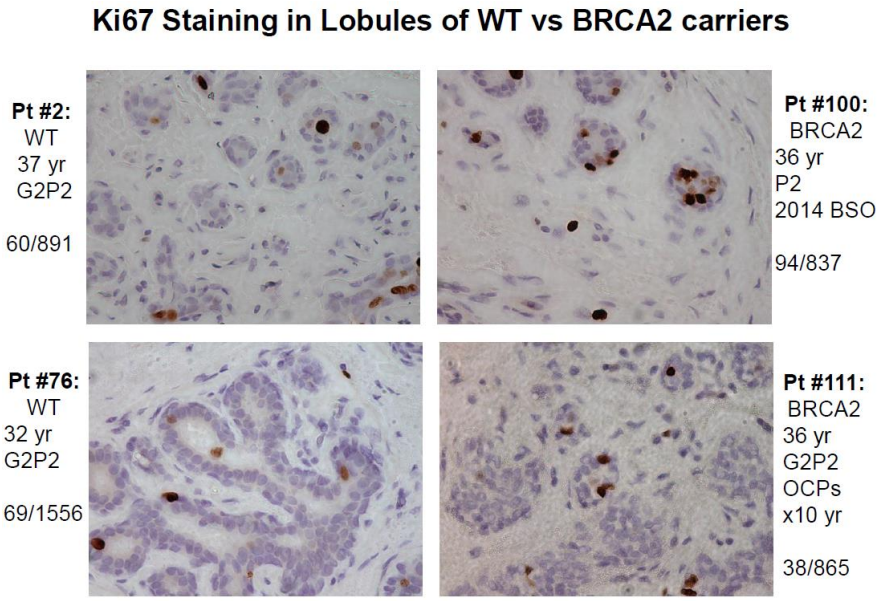
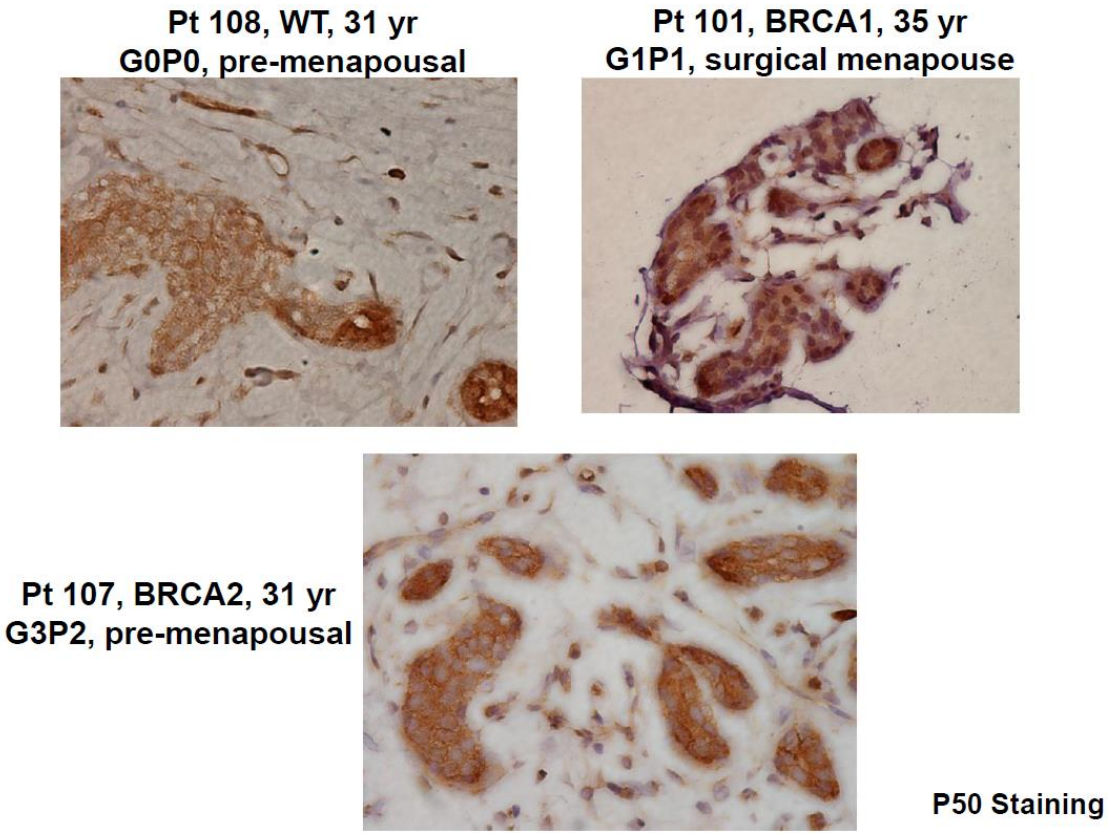


Figure below shows representative staining for the NF-KB subunit p50 in WT, BRCA1 and BRCA2 carrier mammary epithelia in vivo. Clinical characteristics of each subject are indicated. Activated NF-KB is evidenced by nuclear staining, most prominent in a BRCA1 carrier.

NF-KB p50 Staining in lobules of WT, BRCA1 and BRCA2 carriers



Accomplishments: Figure 5 demonstrate a trend toward increased Ki67 staining in luminal cells from BRCA2 carriers, which is consistent with other assays described above and supports the overall hypothesis of deregulated proliferation in luminal cells of BRCA2 carriers. Figure 6 suggests high NF-KB activity in BRCA1 carriers, which is consistent with prior publications. We show for the first time that BRCA2 carriers do not exhibit this elevated NF-KB signaling, which has important implications for cancer prevention approaches in these patients.

Specific Aim 2: Discover and validate new pathways activated in cancer-predisposed tissues.
Major Task 1: Transcriptomic analysis in LP and MASC cells
Methods and accomplishments: (Figure 2 below, carried out during months 1-24).

Methods: We have now carried out additional RNA sequencing on RNA extracted from primary FACS-sorted mammary epithelial cells during months 12-24. Additional comprehensive genomic analyses have been carried out, from control (N=9), BRCA1 carriers (N=7) and BRCA2

carriers (N=7) to date. In each case, basal cells, LP cells, mature luminal cells and stromal cells were analyzed separately.

Accomplishments: The most biologically and clinically significant finding to date (Figure below) is that, unlike BRCA1-mutant mammary epithelia, BRCA2 mutant epithelia exhibit highly suppressed NF-KB signaling. The second important observation (Figure 3) is a cell cycle stress ("G1/G2") RNA profile in BRCA2 mammary LP cells (See Below).

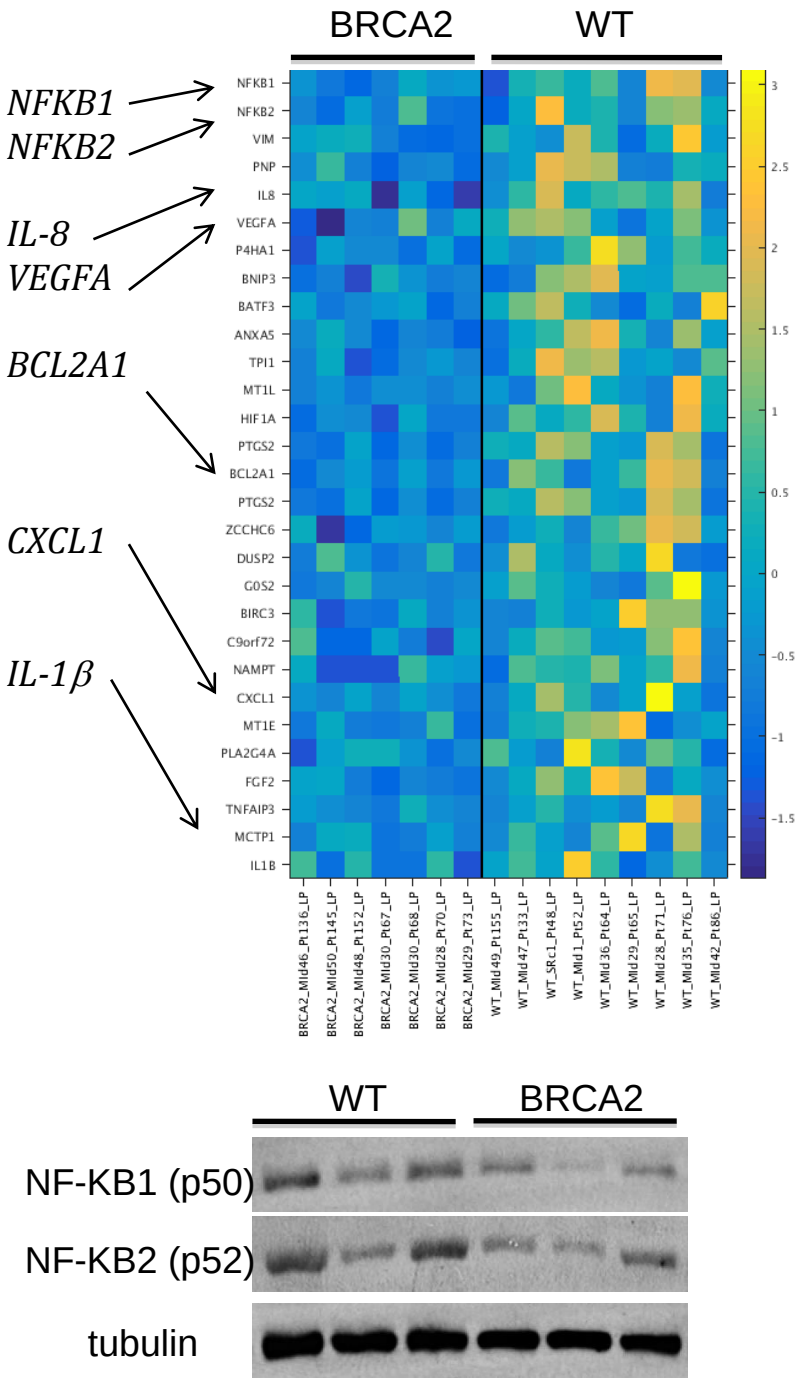
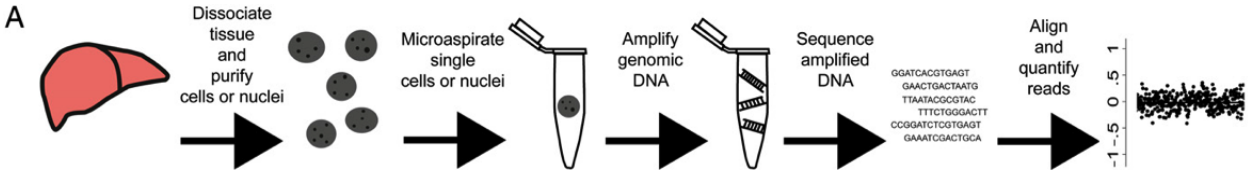


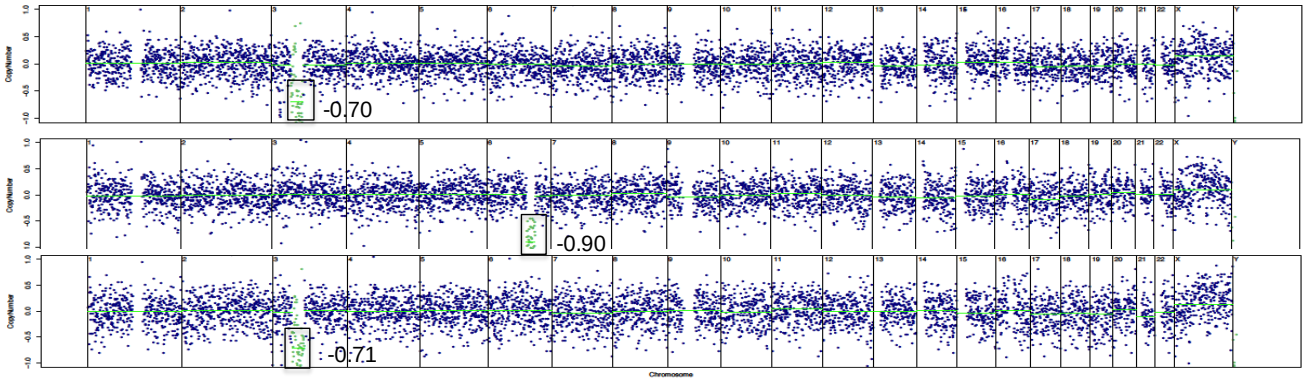
Figure (left) Legend: Heat map of NFkB signature. Each column represents one LP sample in BRCA2 (left) vs WT (right) patient sample. Rows represent the genes in NF-KB pathway. Arrows indicate direct NF-KB target genes. Blue color indicates down- regulation and yellow indicates up-regulation. Below represents western blot analysis of primary breast tissues in WT vs BRCA2 carrier patients.

Major Task 2. Sub-clonal genomic analysis in LP and MASCs

Methods: We carried out low-coverage whole-genome sequencing on individual LP and Basal/MASC cells from primary tissues of BRCA2 carriers during months 12-24. Bioinformatic analysis was carried out to determine genomic quantification and copy number. The experimental protocol is shown below:



Accomplishments: these analyses provided an unprecedented window in genomic aberrations in early breast cancer pathogenesis. In brief, we showed that 30% of LP cells from BRCA2 carriers demonstrate significant chromosomal aneuploidy, compared to <5% of LP cells from wild-type tissues. This is the first demonstration of such aneuploidy in normal tissues in the setting of BRCA1/2 mutation.



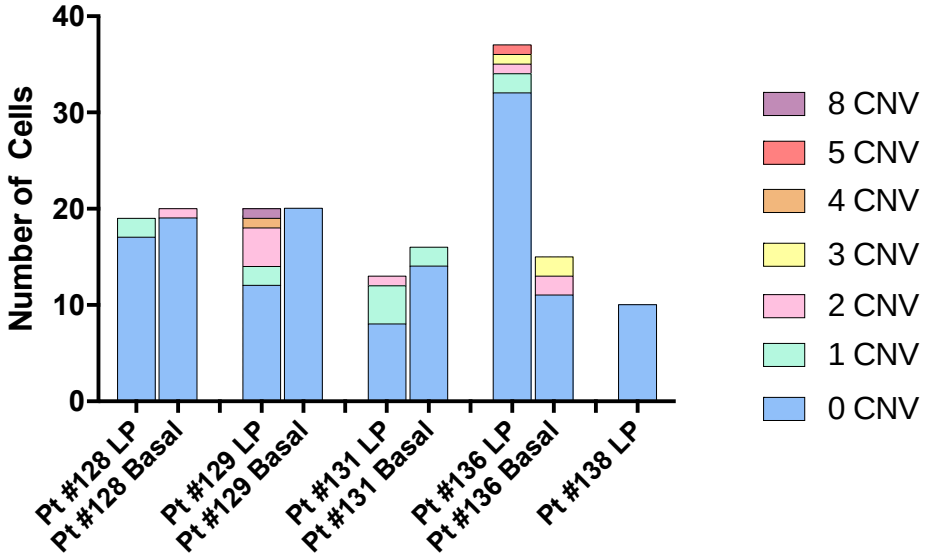


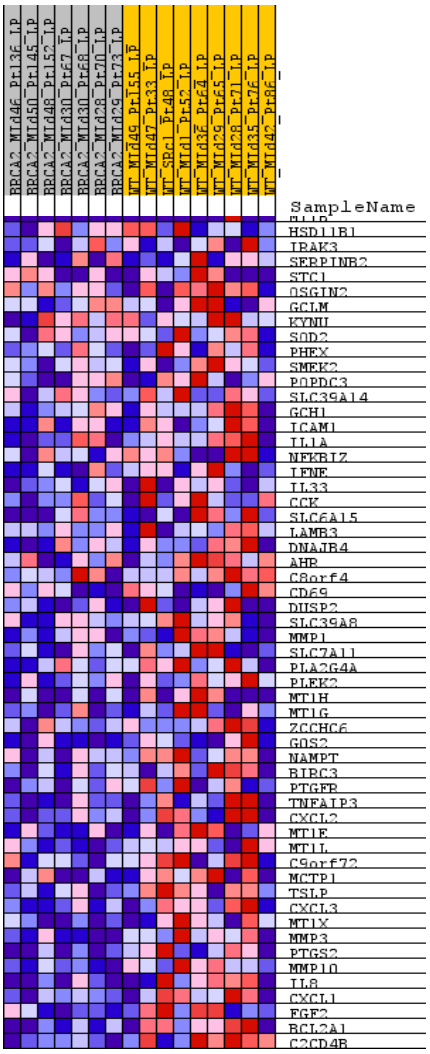
Figure 3. Above shown is whole-genome analysis of cells from a BRCA2 carrier. In the above figure each horizontal row is one cell (3 cells shown), while each box within the row represents an individual chromosome in that cells. Normal DNA content is indicated by dots (sequencing reads) aligned horizontally in the center of each box. Loss of a portion of the chromosome is evidenced by dots below the center line, which are shown in green. Thus, each cell shown has on major sub-chromosomal loss involving a different chromosome. Below shown is the summary of CNVs identified in LP and basal cells of all BRCA2 carrier patients we sequenced. Different colors represent the number of CNVs identified in each cell.

Major Task 3: Integrated bioinformatics of expression/mutation (ONGOING)

Methods: Together with our computational biologists, we have integrated the genomic findings with the gene expression data to identify signatures associated with the aneuploid state we discovered.

Accomplishments: Current comprehensive integrated analysis shows a gene expression signature of DNA damage suppression that is likely a reflection of why these cells survived despite having a high aneuploidy state. This may prove to be an "achilles heel" of the BRCA2 mutant tissue that can be exploited to eliminate abnormal, aneuploid cells that are the precursors of breast cancer in this genetic context.

BRCA2 WT



BRCA2_LP vs. WT_LP

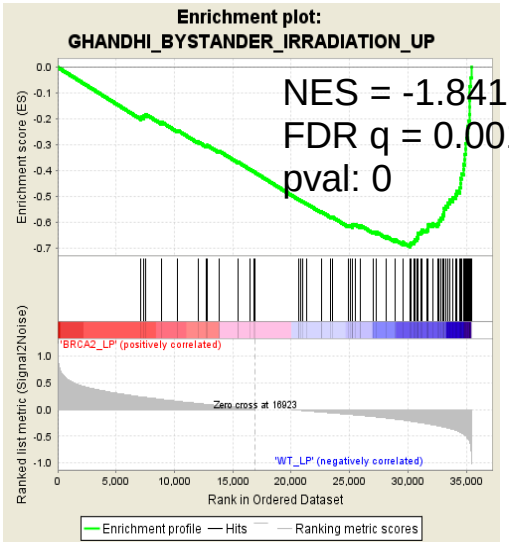


Figure 4. Heatmap and GSEA plot of a DNA damage response signature shows suppressed DNA damage response in BRCA2 carrier patients.

Specific Aim 3: Block abnormal signaling in vitro and in vivo

Major Task 1. Reverse abnormal LP signaling in vitro (ONGOING)

Methods: Given our observation of aneuploidy in BRCA2-mutant cells from patients, we sought to exploit this abnormal DNA damage phenotype as a potential cancer prevention strategy. Accordingly, we treated performed comet assay using primary epithelia. We will induce NF-KB pathway with TNF induction to see if this phenotype reverses.

Accomplishments: These experiments demonstrate accumulated DNA damage in BRCA2 cells. These experiments thus provide proof-of-principle for use of this and related agents to eliminate breast cancer precursor cells as a prevention strategy for breast cancer in young women.

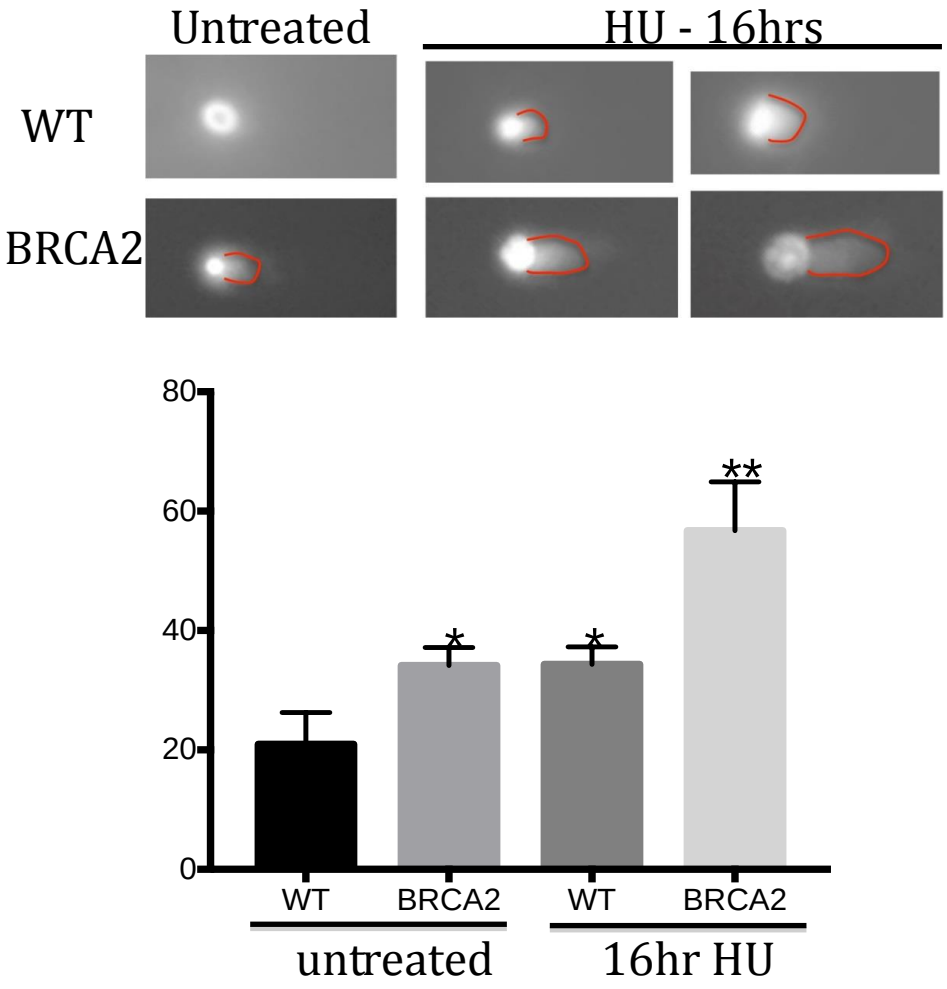


Figure 5. Comet assay was applied using uncultured BRCA2-mutant and WT primary epithelia with HU treatment. BRCA2 cells have more DNA damage which increases with replication stress.

Major Task 2: Inhibit abnormal signaling in vivo cancer models

(IN PROCESS). Note that animal experiments are planned in months are temporarily delayed due to capacity issues in our institutional animal facility. We anticipate commencement of these studies in months 24-36.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Mihriban Karaayvaz, PhD is providing ongoing training to Devika Salunke, a research assistant in the laboratory who is developing professional skills in preparation for graduate school.

Dr. Karaayvaz herself was able to attend a bioinformatics course, as well as multiple conferences concerning topics related to the area of the proposal.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

1. Presentation at the Harvard Cancer Center Breast/Ovarian Cancer Retreat 3/2017.
2. Presentation at the Harvard Cancer Center Breast/Ovarian Cancer Retreat 3/2017.
3. Presentation at the Gordon Research Conferences, 2018 March 24-25; Ventura, CA
4. See Appendix for full list of abstracts and presentations.
5. A major manuscript describing these findings will be submitted for peer review by 9/2018.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Additional integrated bioinformatic analysis of RNAseq and genomic data were completed. Furthermore, we are continuing testing of functional pathways and hypotheses gleaned from the bioinformatic analysis.

These experiments will set the stage for ongoing experiments in Aim 3, to unveil unanticipated synthetic lethalities in BRCA1 and BRCA2 mutant non-malignant cells. These studies will set the stage for in vivo tests of blocking these pathways in order to credential new opportunities for prevention of breast cancers in young women.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

New types of techniques for human breast tissue analysis described in the figures above, and functional characterization are being developed through this project, and these will allow other investigators to explore related questions in breast cancer biology.

1. New findings have provided unprecedented view into early cancer pathogenesis in humans. A major manuscript describing these findings will be submitted for peer review by 9/2018.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The concept that sub-chromosomal aneuploid events occur at the earliest stages of cancer pathogenesis has implications for human development and disease far beyond breast cancer.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

It is anticipated that these remarkable findings will pave the way for further exploration of the earliest steps in cancer pathogenesis, leading to the development of novel methodologies for early cancer detection and revolutionary approaches for cancer prevention. We continue to actively pursue this area of investigation with a goal of developing potentially commercially applicable approaches.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Use of donated tissue proves the value of this approach for scientific advances that benefit patients. This concept will be disseminated through the results of this research.

5. CHANGES/PROBLEMS: The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

None to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Delays in implementation of animal experiments as described above due to institutional limitations.

expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None.

Significant changes in use of biohazards and/or select agents

None.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

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

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Saladi SV**,** Ross K, **Karaayvaz M**, Tata PR, Hongmei M, Rajagopal J, Ramawamy S, Ellisen LW. ACTL6A is co-Amplified with p63 to Drive YAP Activation, Regenerative Proliferation and Poor Prognosis. **Cancer Cell**. (2017); (31): 35-49. Federal support is acknowledged.
A major manuscript describing our findings will be submitted for peer review by 9/2018.
Other publications in press/in preparation are listed in the Appendix.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None to date.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. Presentation in joint laboratory meeting, MGH Center for Cancer Research.
2. Presentation at Scientific Advisory Board Meeting of the Mass General Hospital
3. Presentation at the Harvard Cancer Center Breast/Ovarian Cancer Retreat.
4. Presentation at the MGH Cancer Center Annual Retreat.
5. See Appendix for complete abstracts and presentation list.

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Our laboratory web site gives a description of this ongoing work and a link to all publications and personnel.

https://www.massgeneral.org/cancerresearch/research/researchlab.aspx?id=1167

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

New types of techniques for human breast tissue analysis and functional characterization are being developed through this project, and these will allow other investigators to explore related questions in breast cancer biology.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None to date.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*

- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

New datasets of gene expression in normal and mutated human breast tissue will be a valuable resource and will be publicly available once results of the study are published.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Leif Ellisen*
Project Role:	*PI*
Nearest person month worked:	*1*
Contribution to Project:	*No change*
Funding Support:	*No change*
Name:	*Devika Salunke*
Project Role:	*Research Assistant*
Nearest person month worked:	*12*
Contribution to Project:	*No change*
Funding Support:	*No change*
Name:	*Kenneth Ross*
Project Role:	*Bioinformatician*
Nearest person month worked:	*1*
Contribution to Project:	*No change*
Funding Support:	*No change*
Name:	*Mihriban Karaayvaz*
Project Role:	*Research Fellow*
Nearest person month worked:	*6*
Contribution to Project:	*No change*
Funding Support:	*No change*

No change in active support for PI/key personnel.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

The project is conducted at the Mass General Hospital.
A portion of cell sorting is conducted at the Ragon Institute of MGH, MIT and Harvard.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award. **N/A**

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments. **N/A**

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Attached: Presentations, Abstracts and Work Product During the Funding Period

APPENDIX

Presentations and Work Product During the Funding Period

Poster Presentations

Karaayvaz M, Ross K, Saladi S, Silberman RE, Langenbucher A, Zarcaro E, Desmond A , Ravichandran H, Mylavagnanam R, Specht MC, Lawrence M, Amon A, Ellisen LW. Altered Progenitor Function Define Early Pathogenesis of BRCA2-associated Breast Cancer; Gordon Research Conferences, 2018 March 24-25; Ventura, CA

Karaayvaz M, Ross K, Saladi S, Silberman RE, Langenbucher A, Zarcaro E, Desmond A , Ravichandran H, Mylavagnanam R, Specht MC, Lawrence M, Amon A, Ellisen LW. Altered Progenitor Function Define Early Pathogenesis of BRCA2-associated Breast Cancer; Dana-Farber Harvard Cancer Center (DFHCC) Breast and Gynecologic Cancer Symposium; 2018 March 16; Boston, MA

Karaayvaz M, Ross K, Desmond A, Specht MC, and Ellisen LW. Altered Progenitor Function and Breast Cancer Predisposition. Dana-Farber Harvard Cancer Center (DFHCC) Breast and Gynecologic Cancer Symposium; 2017 February 8; Boston, MA

Karaayvaz M, Ross K, Desmond A, Specht MC, and Ellisen LW. Altered Progenitor Function and Breast Cancer Predisposition. Mass General Hospital Cancer Center Retreat; 2016 September 22-23; Boston, MA

Karaayvaz M, Ross K, Desmond A, Specht MC, and Ellisen LW. Altered Progenitor Function and Breast Cancer Predisposition. Dana-Farber Harvard Cancer Center (DFHCC) Breast and Gynecologic Cancer Symposium; 2016 March 3; Boston, MA

Karaayvaz M, Specht MC, and Ellisen LW. Altered Progenitor Function and Breast Cancer Predisposition. Dana-Farber Harvard Cancer Center (DFHCC) Breast and Gynecologic Cancer Symposium; 2015 March 7; Boston, MA

Karaayvaz M, Specht MC, and Ellisen LW. Altered Progenitor Function and Breast Cancer Predisposition. MGH Office for Research Career Development (ORCD) Research Fellows Poster Celebration; 2015 May 27; Boston, MA

Karaayvaz M, Ross K, Specht MC, Ellisen LW. Altered Progenitor Function and Breast Cancer Predisposition. In Mechanisms and Models of Cancer Meeting of Salk Institute, 2015 August 5-8; La Jolla, CA

Oral Presentations

Aneuploidy and altered cell fate define early pathogenesis of BRCA2-associated breast cancer. Biennial International BRCA1/2 Conference, May 8-11, Montreal, Canada

Unravelling subclonal heterogeneity and aggressive disease states in TNBC through single cell RNA-seq. Mass General Hospital Cancer Center, 2018 February 23, Boston, MA

Altered Cell Fate and Signaling Distinguish Early Cancer Pathogenesis for BRCA1 *vs* BRCA2. Mass General Hospital Cancer Center, 2017 February 24, Boston, MA

Altered Progenitor Function and Breast Cancer Predisposition. Dana-Farber Harvard Cancer Center (DFHCC) Breast and Gynecologic Cancer Symposium; 2016 March 3; Boston, MA

Altered Progenitor Function Define Early Pathogenesis of BRCA2-associated Breast Cancer. 4nd Turkish Medicine Council; 2017 October 28-29; Istanbul, Turkey

Publications

Saladi SV**,** Ross K, **Karaayvaz M**, Tata PR, Hongmei M, Rajagopal J, Ramawamy S, Ellisen LW. ACTL6A is co-Amplified with p63 in Squamous Cell Carcinoma to Drive YAP Activation, Regenerative Proliferation and Poor Prognosis. **Cancer Cell**. (2017); (31): 35-49

Karaayvaz M[*], Cristea S[*], Gllespie SM, Patel AP, Mylvaganam R, Luo CC, Specht MC, Bernstein BE, Michor F, Ellisen LW. Unravelling subclonal heterogeneity and aggressive disease states in TNBC through single cell RNA-seq. Nature Communications; (2018) (In press).
[*] These authors contributed equally.

Karaayvaz M, Langenbucher A, Saladi SV, Ross KN, Silberman RE, Zarcaro E, Desmond A, Ravichandran H, Mylavagnanam R, Specht,MC, Lawrence M, Amon A, Ellisen LW. Aneuploidy and altered cell fate define early pathogenesis of BRCA2-associated breast cancer (2018) (In preparation).

Abstract for International Montreal BRCA1/2 Meeting 2018:

Germline mutations in BRCA1 and BRCA2 confer risks for distinct breast cancer subtypes, as most BRCA1-associated cancers are estrogen receptor (ER) negative and “basal-like”, whereas those arising in BRCA2 genetic carriers are predominately ER positive (ER+) and “luminal-like”. The biological basis for these divergent phenotypes remains unknown. We thus sought to reveal the earliest steps in BRCA1/2-associated cancer pathogenesis through detailed cellular, molecular and functional analysis of FACS-sorted cell populations from freshly isolated, histologically normal tissues of BRCA1/2 carriers and matched controls. We observe an age-associated increase in the luminal progenitor (LP) population and increased luminal/basal cell ratio selectively in BRCA2 carriers. Correspondingly, RNAseq analysis demonstrates dramatic suppression of NF-kb signaling, a key determinant of mammary cell fate, in the LP compartment of BRCA2 carriers compared to BRCA1 carriers and controls, associated with down-regulation of genes signifying basal and bipotent cell fate. Furthermore, we have uncovered a prominent signature of G2/M stress and Aurora B kinase activation selectively in LP cells of BRCA2 carriers, which is linked to DNA damage as evidenced by increased g-H2-AX staining in the corresponding tissues. Correspondingly, we employed a validated next-generation sequencing (NGS)-based methodology for single-cell ploidy analysis, which demonstrates polyclonal aneuploidy in >20% of BRCA2 LP cells, including large (>10MB) deletions never observed in normal cells. We conclude that the early pathogenesis of BRCA2-associated breast cancer involves altered NF-kb signaling and the resulting expansion of a luminal fate-shifted progenitor population that is subject to mitotic stress, triggering Aurora kinase activation and aneuploidy that ultimately drives malignant progression. These findings have important implications for breast cancer prevention and pathogenesis in BRCA1/2 genetic carriers.

Abstract for other BRCA1/2 meetings:

Germline mutations in BRCA1 and BRCA2 confer risk for distinct breast cancer subtypes, as most BRCA1-associated cancers are estrogen receptor (ER) negative and basal-like, whereas those arising in BRCA2 genetic carriers are predominately ER positive (ER+) and luminal-like. The biological basis for these divergent phenotypes remains unknown. We thus sought to reveal the earliest steps in BRCA1/2-associated cancer pathogenesis through detailed cellular, molecular and functional analysis of FACS-sorted cell populations from freshly isolated, histologically normal tissues of BRCA1/2 carriers and matched controls. We observe an age-associated increase in the luminal progenitor (LP) population and increased luminal/basal cell ratio selectively in BRCA2 carriers. Correspondingly, RNAseq analysis demonstrates dramatic suppression of NF-kb signaling, a key determinant of mammary cell fate, in the LP compartment of BRCA2 carriers compared to BRCA1 carriers and controls, associated with down-regulation of genes signifying basal and bipotent cell fate. Furthermore, we

have uncovered a prominent signature of G2/M stress and Aurora B kinase activation selectively in LP cells of BRCA2 carriers, which is linked to DNA damage. We conclude that the early pathogenesis of BRCA2-associated breast cancer involves altered NF-kb signaling and the resulting expansion of a luminal fate-shifted progenitor population that is subject to mitotic stress, triggering Aurora kinase activation that ultimately drives malignant progression. These findings have important implications for breast cancer prevention and pathogenesis in BRCA1/2 genetic carriers.

Abstract for related to BRCA and TNBC:

Triple-negative breast cancer (TNBC) is an aggressive subtype characterized by extensive intratumoral heterogeneity. While many TNBCs undergo substantial regression following chemotherapy, a failure to eradicate all viable primary tumor cells is associated with a high probability of metastatic relapse and death, suggesting that a minor subpopulation of tumor cells drives these poor outcomes. To investigate the underlying biology, we conducted single-cell RNA sequencing (scRNA-seq) of >1500 cells from six primary TNBC. Intercellular heterogeneity of gene expression programs within each tumor was variable and largely correlated with clonality of inferred genomic copy number changes, suggesting that genotype drives the gene expression phenotype at the level of individual subpopulations. Clustering of gene expression profiles identified distinct subgroups of malignant cells shared by multiple tumors, including a single subpopulation associated with multiple signatures of treatment resistance and metastasis, and characterized functionally by activation of glycosphingolipid metabolism and associated innate immunity pathways. A novel signature defining this subpopulation was predictive of long-term outcomes for TNBC patients in a large patient cohort. Collectively, this analysis reveals the functional heterogeneity and its association with genomic evolution in TNBC and uncovers unanticipated biological principles dictating poor outcomes in this disease.