AWARD NUMBER: W81XWH-15-2-0006

TITLE: Mechanisms of Resistance to Chemotherapies Targeting BRCA-Mutant Breast Cancer

PRINCIPAL INVESTIGATOR: Dr. Andre Nussenzweig

CONTRACTING ORGANIZATION:
The Geneva Foundation

Tacoma, WA 98402

REPORT DATE: FEB 2019

TYPE OF REPORT: Final Report

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
FEB 2019	Final Report	01 Dec 2014 -30 Nov 2018

4. TITLE AND SUBTITLE
Mechanisms of Resistance to Chemotherapies Targeting BRCA-Mutant Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-2-0006

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Andre Nussenzweig, Ph.D.

E-Mail: nussenza@exchange.nih.gov

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

The Geneva Foundation
917 Pacific Ave. #600
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
FORT DETRICK MD 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

This research proposal for the BRCP Idea Expansion Award involves a detailed study of the underlying mechanisms of drug resistance in BRCA1 and BRCA2 deficient cells through the genetic manipulation of the major players that mediate homologous recombination and non-homologous end joining DNA repair pathways. These studies will serve as a foundational data set in determining the importance of DNA repair pathways in acquiring resistance to breast and ovarian cancer chemotherapies. Because drug resistance is an inescapable feature of all cancer treatments, an understanding of the causal mechanisms of resistance will undoubtedly provide new opportunities and susceptible targets in cancer therapies.

15. SUBJECT TERMS

NONE LISTED

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

1. INTRODUCTION:

In ongoing work being performed in support of the BCRP Idea Expansion Award, we have hypothesized that restoration of homologous recombination (HR) is a critical driver of chemo-resistance in BRCA1/2 mutated breast cancers. Earlier work provided evidence for a novel mechanism of HR restoration, through the inactivation of proteins functioning in an alternate DNA repair pathway called non-homologous end joining (NHEJ). Loss of NHEJ proteins, such as 53BP1, restored normal HR activity in BRCA1 deficient cells and rendered these cells resistant to chemotherapeutic agents. Under the aegis of the Idea Expansion Award, we have discovered a novel and unexpected mechanism of drug resistance in BRCA-deficient cancers that involves the loss of the 53BP1-interacting protein PTIP that operates independently of the re-establishment of HR. Overall, the major aim of this research is to understand the manifold routes that lead to acquired resistance in breast cancer treatment and how altering the balance between NHEJ, HR and other pathways can be exploited to overcome the Achilles heel of acquired resistance.

2. KEYWORDS

1. 53BP1: p53 Binding Protein 1
2. Brca1 & 2: Breast cancer type 1 & 2 susceptibility protein, tumor suppressor
3. BRCT domain: BRCA1 Carboxy Terminal Domain
4. CD19: Cluster of Differentiation 19
5. CRE: Causes Recombination
6. DSB: Double Strand Breaks
7. H2AX- a variant of Histone 2A, a core chromatin protein HR: Homologous Recombination
8. MEFs: Mouse Embryonic Fibroblasts
9. NHEJ: Non-Homologous End Joining
10. PA1: PTIP-Associated protein 1
11. PALB2: PArtner and Localizer of BRCA2
12. PARP: Poly ADP-Ribose Polymerase
13. PARPi: Poly ADP-Ribose Polymerase Inhibitor
14. PTIP: Pax Transactivation Domain-Interacting Protein
15. RAP80: Receptor-Associated Protein 80
16. RIF1: RAP1 Interacting Factor 1
17. RNF8: Ring Finger protein 8
18. RNF168: Ring Finger protein 168
19. RPA: Replication Protein A
20. TCGA: The Cancer Genome Atlas

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the USAMRAA Grants Officer whenever there are significant changes in the project or its direction.

- What were the major goals and objectives of the project?
- What was accomplished under these goals?
- What opportunities for training and professional development did the project provide?

- How were the results disseminated to communities of interest?

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

What were the major goals of the project?

In this proposal, we outlined two major Specific Aims and Goals. In **Specific Aim 1**, we proposed the use of a genetic approach to define interactions between NHEJ and HR effectors that regulate DNA repair pathway selection. In specific **Sub-Aim 1a**, we intend to test the hypothesis that elevated levels of chromatin bound 53BP1 converts wild type cells to a 'BRCA-deficient' state in the context of drug sensitivity and genome stability. In specific **Sub-Aim 1b**, the importance of PTIP associated proteins (PA1 and MLL4) and the functional domains within PTIP necessary for HR reconstitution are to be evaluated by *in vivo* experimentation.

In **Specific Aim 2**, we proposed testing the contribution of the 53BP1 associated protein (PTIP) in influencing repair pathway selection and to also computationally interrogate clinical data sets together with data acquired from murine models to understand the mechanisms of chemo-resistance in BRCA1- and BRCA2-mutated cancers. In specific **Sub-Aim 2a**, we are testing the hypothesis that PTIP can influence repair pathway selection and drug sensitivity. In specific **Sub-Aim 2b**, we plan on using gene expression data and other genome-wide data sets from clinical databases to implicate known (and novel) proteins involved in the regulation of chemoresistance.

What was accomplished under these goals?

A. RNF168 promotes a BRCA1-independent alternative HR pathway that maintains genome stability in BRCA1-deficient cells

In our 2016 Annual Report, we addressed whether mechanisms other than restoration of homologous recombination (HR) can lead to the development of chemoresistance in BRCA1/2-deficient tumors (**Specific Aim 2**). In our 2017 Annual Report, we provided the first example of a genetic alteration that unmasks BRCA1 haploinsufficiency, and demonstrates the important function of RNF168 as an alternative PALB2 recruiting factor in BRCA1 compromised cells. This is an important mechanism by which cells with germline heterozygous BRCA1 mutations employ to maintain genome integrity and chemoresistance (**Specific Aim 1**). We proposed that while BRCA1 deficient tumors can become chemoresistant by losing 53BP1, their reliance on RNF168 presents an excellent pharmacological target and a means to re-sensitize previously resistant tumor cells.

The work leading to the conclusions detailed in the previous annual report and new observations that further validate these findings are described below in the Accomplishments section of the 2018 annual report and is now in press (2019):
'***BRCA1 haploinsufficiency is masked by RNF168-mediated chromatin Ubiquitylation***'

Dali Zong, Salomé Adam, Yifan Wang, Hiroyuki Sasanuma, Elsa Callén, Matilde Murga, Amanda Day, Michael J. Kruhlak, Nancy Wong, Meagan Munro, Arnab Ray Chaudhuri, Baktiar Karim, Bing Xia, Shunichi Takeda, Neil Johnson, Daniel Durocher, André Nussenzweig
Molecular Cell (2019, in press)

A. BRCA1 haploinsufficiency is masked by RNF168-mediated chromatin ubiquitylation

BRCA1 plays dual roles in HR both by potentiating DNA end-resection and by subsequently delivering RAD51 onto 3' single stranded DNA (ssDNA) substrates. Although its function in end-resection remain unclear, BRCA1 may act in part by removing the end-blocking factor 53BP1 from chromatin surrounding DNA double strand breaks (DSBs), which enables access and long-range resection by the DNA end-processing machinery. In addition, BRCA1 interacts with PALB2 through its coiled-coiled domain, bridging it with BRCA2 post-resection, which in turn, promotes assembly of RAD51 onto 3' ssDNA. Since inactivation of 53BP1 stimulates end resection and HR proficiency in BRCA1- but not in BRCA2- deficient cells, it has been assumed that loss of 53BP1 bypasses the downstream role of BRCA1 in loading RAD51. However, whereas genomic instability and embryonic lethality is rescued in BRCA1-deficient mouse cells, deletion of 53BP1 exacerbates genome instability in PALB2 knockout cells, suggesting that BRCA1-deficient cells are capable of initiating an alternative mode of RAD51 loading when end-resection is restored. In the data shown below, we demonstrate that the RNF168-mediated chromatin ubiquitylation pathway acts redundantly with BRCA1 to promote PALB2- and RAD51-dependent HR. Moreover, RNF168 activity is essential to prevent overt genome instability and tumorigenesis in BRCA1 heterozygous mice, independently of p53 mutation. This leads to the suggestion that the unmasking of BRCA1 haploinsufficiency by RNF168 deregulation may contribute to tissue-specific cancer predisposition in BRCA1 mutations carriers.

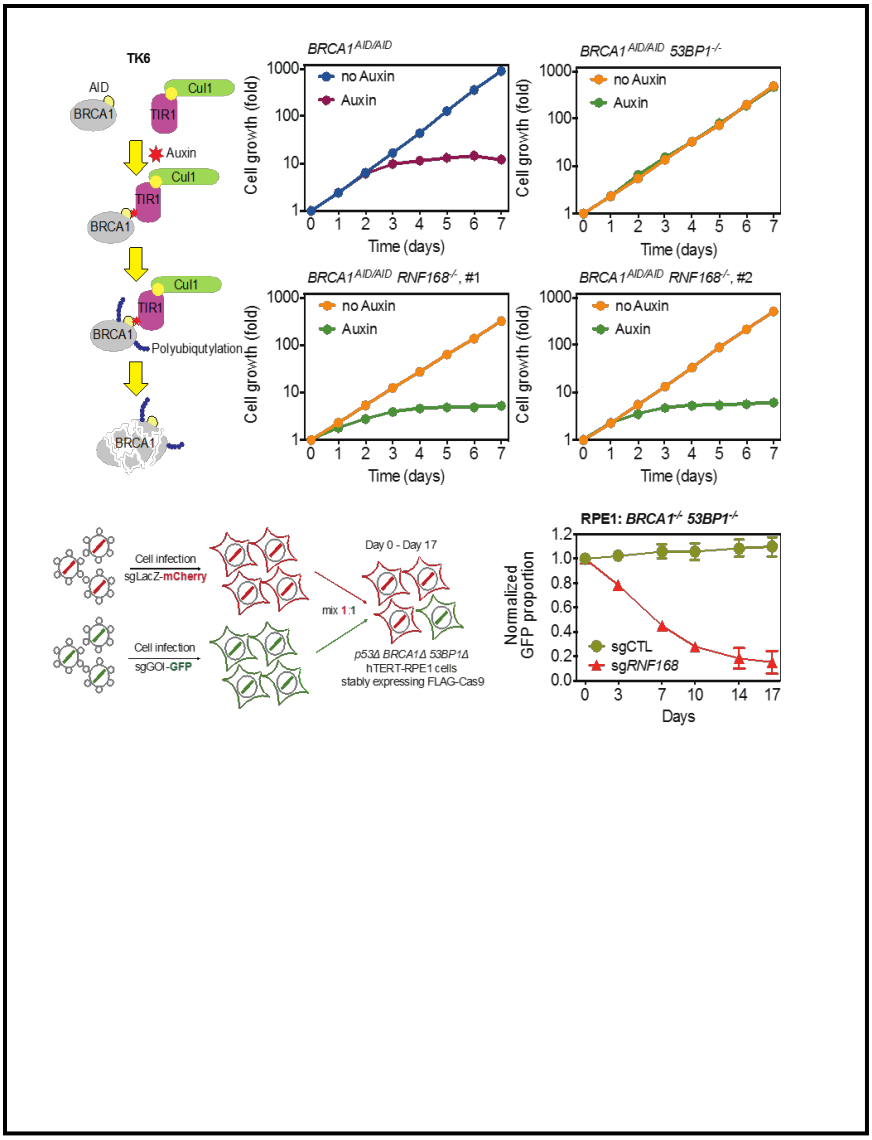
The work leading to the conclusions detailed above forms the basis of a manuscript entitled "BRCA1 haploinsufficiency is masked by RNF168-mediated chromatin ubiquitylation". This manuscript is in press in *Molecular Cell* (2019).

The Significant Results and Key Outcomes summarized below highlight data germane to **Specific Aim 1** outlined in this proposal

A1. **RNF168 supports BRCA1-independent HR in human cells**

Work described in our 2017 Annual Report demonstrated a crucial role for RNF168 in promoting BRCA1-independent HR in mice. However, conflicting observations have been made in human cells. siRNA depletion of RNF168 was reported to suppress the HR defect caused by BRCA1 silencing in human cells, while cells co-depleted of BRCA1, 53BP1 and RNF8 showed a reduction in RAD51 foci formation. To definitively compare the impact of RNF168 vs. 53BP1 loss in human cells lacking BRCA1 and avoid potential confounding factors arising from hypomorphic BRCA1 alleles, we took advantage of an auxin-based degron system in which

BRCA1 protein can be rapidly and conditionally depleted in human TK6 cells (**Fig. 1**, **Top Left Panel -** schema). Acute depletion of human BRCA1 resulted in a rapid cessation of proliferation followed by cell death, which was accompanied by a loss of capacity to form RAD51 foci (**Fig. 1**, **Top Right Panels**). In accord with our previous mouse studies, deletion of 53BP1 by CRISPR/Cas9 rescued these phenotypes (**Fig. 1**, **Top Right Panels**). In contrast, deletion of RNF168 failed to restore the growth defect in BRCA1-depleted cells (**Fig. 1**, **Top Right Panels**). Consistent with the observation in TK6, guide RNAs (sgRNA) targeting RNF168 was able to reverse the PARPi resistant phenotype of human RPE1 cells in which both BRCA1 and 53BP1 had been deleted using CRISPR-Cas9 (**Fig. 1**, **Bottom Left and Right Panels**).

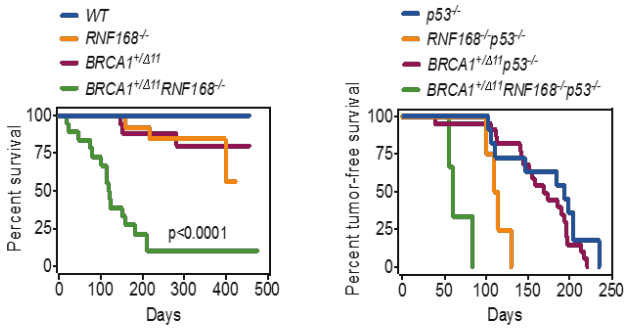


Thus, RNF168 is required to support BRCA1-independent survival and RAD51 foci formation in both mouse and human cells.

A2. RNF168 deletion reveals BRCA1 haploinsufficiency

Mutation of a single BRCA1 allele leads to cancer predisposition, although mouse models of BRCA1 heterozygosity do not show genome instability or tumorigenesis. We had previously shown the severe impact of RNF168 loss in BRCA1$^{\Delta 11/\Delta 11}$ cells, we wished to determine whether RNF168 activity might also be essential in BRCA1 heterozygous cells. BRCA1$^{+/\Delta 11}$ heterozygous mice expressing RNF168 were born at normal frequency and did not exhibit any

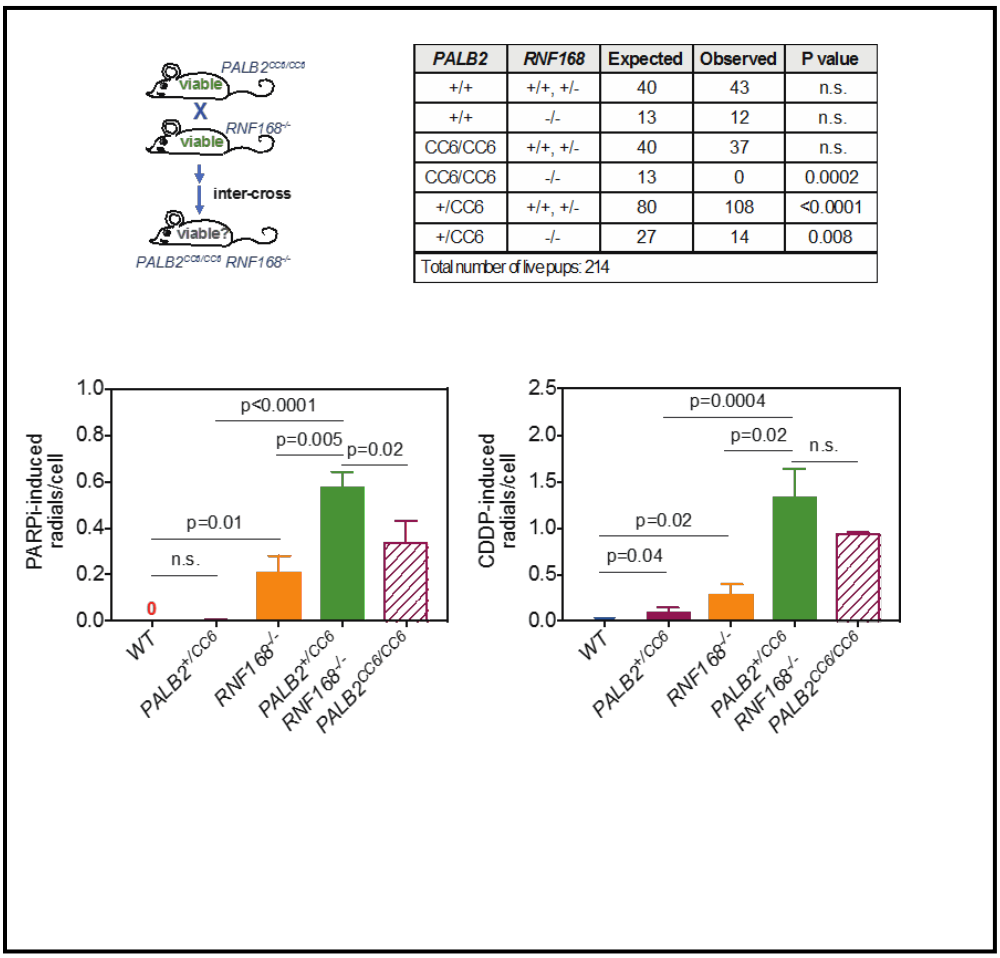
notable phenotypes. However, deletion of RNF168 had a profound impact on the viability of heterozygous BRCA1$^{+/\Delta 11}$ mice. Live BRCA1$^{+/\Delta 11}$RNF168$^{-/-}$ pups were born at significantly sub-Mendelian frequencies, even though both BRCA1$^{+/\Delta 11}$ and RNF168$^{-/-}$ mice were born at normal frequencies (**data shown in 2017 Annual Report**). We conclude that RNF168 loss reveals latent defects associated with BRCA1 heterozygosity. Despite the fact that BRCA1$^{+/\Delta 11}$RNF168$^{-/-}$ pups were born at sub-Mendelian frequencies, we eventually obtained a cohort of live pups through extensive breeding (19 out of 68 expected, total n=727, p<0.0001) but they were consistently smaller than their BRCA1$^{+/\Delta 11}$ littermates. BRCA1$^{+/\Delta 11}$RNF168$^{-/-}$ mice exhibited significantly shortened lifespan with a median survival of 120 days, as compared to either BRCA1$^{+/\Delta 11}$ or RNF168$^{-/-}$ single mutant littermates (343 and 372 days, respectively) (**Fig. 2**, **Top Left Panel**). Moreover, 9 out of 19 BRCA1$^{+/\Delta 11}$RNF168 mice spontaneously developed lymphoma (**Fig. 2**, **Bottom Panel**) Loss of RNF168 also accelerated tumorigenesis in both BRCA1$^{+/\Delta 11}$p53$^{-/-}$ mice (**Fig. 2**, **Top Right Panel**). Thus, while p53 deficiency alone does not foster BRCA1 haploinsufficiency for tumor formation, BRCA1 heterozygous mice become tumor prone when RNF168 is lost.



Mouse ID	BRCA1, RNF168	Age at death (days)	Cancer
4910	+/Δ11, -/-	151	N/D
6273	+/Δ11, -/-	23	N/D
6482	+/Δ11, -/-	159	lymphoma
6499	+/Δ11, -/-	alive (475)	N/D
6623	+/Δ11, -/-	47	N/D found dead
7002	+/Δ11, -/-	20	N/D
7067	+/Δ11, -/-	74	N/D
7222	+/Δ11, -/-	120	lymphoma brain tumor
7271	+/Δ11, -/-	183	lymphoma
7272	+/Δ11, -/-	209	lymphoma
7376	+/Δ11, -/-	100	lymphoma
7471	+/Δ11, -/-	79	N/D
7493	+/Δ11, -/-	113	lymphoma
7513	+/Δ11, -/-	alive (199)	N/D
7692	+/Δ11, -/-	112	lymphoma
7705	+/Δ11, -/-	117	lymphoma
7711	+/Δ11, -/-	alive (165)	N/D
7788	+/Δ11, -/-	122	lymphoma
7826	+/Δ11, -/-	18	none
Total number of *BRCA1*$^{+/\Delta 11}$ *RNF168*$^{-/-}$ mice: 19			

Together, these data demonstrate that BRCA1 becomes haploinsufficient for genome maintenance in the absence of RNF168 ubiquitin ligase activity.

A3. BRCA1 independent PALB2 loading requires RNF168



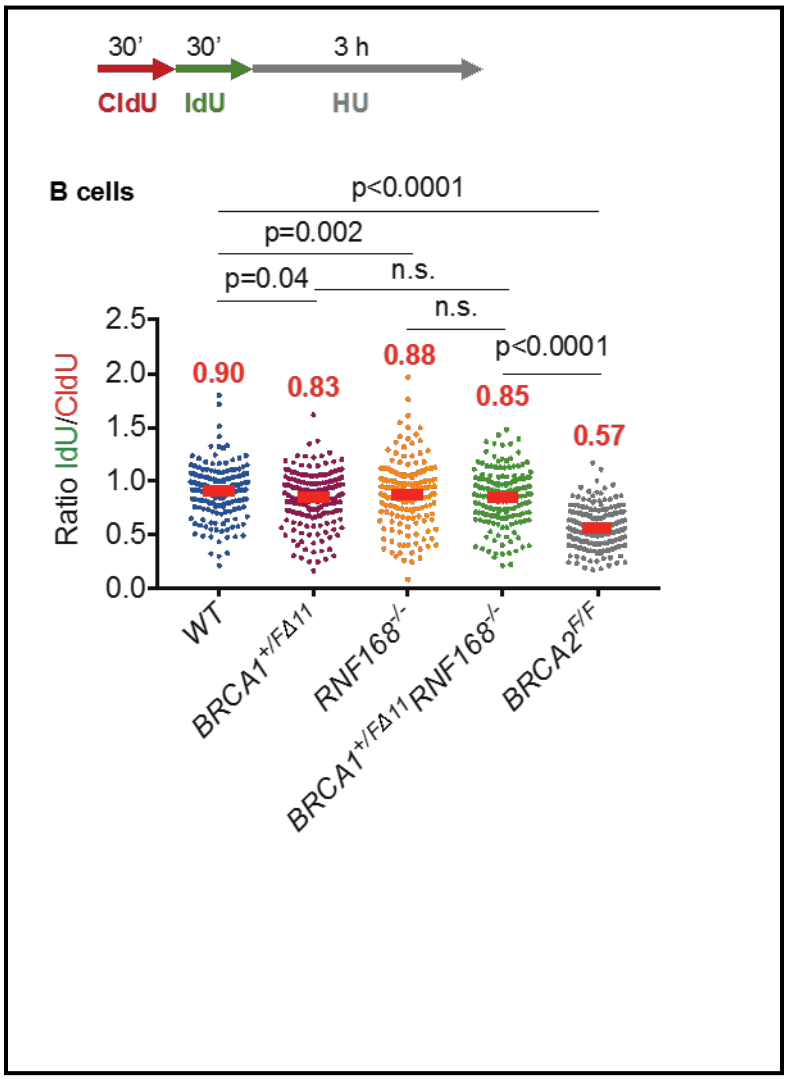
PALB2	RNF168	Expected	Observed	P value
+/+	+/+, +/-	40	43	n.s.
+/+	-/-	13	12	n.s.
CC6/CC6	+/+, +/-	40	37	n.s.
CC6/CC6	-/-	13	0	0.0002
+/CC6	+/+, +/-	80	108	<0.0001
+/CC6	-/-	27	14	0.008
Total number of live pups: 214				

Our data reveals that BRCA1 heterozygous cells and BRCA1$^{-/-}$53BP1$^{-/-}$ cells rely on RNF168 to sustain a critical level of PALB2 recruitment that is sufficient for RAD51-dependent HR and normal growth. To determine whether RNF168-mediated PALB2 recruitment is separable from the canonical BRCA1-dependent PALB2 response, we took advantage of a recent mouse model in which mutations have been introduced into the PALB2 coiled-coil domain to produce a mutant PALB2 protein (PALB2^{CC6}) that is unable to interact with BRCA1 (**Fig. 3, Top Left Panel**). Unlike mice with a complete knockout of PALB2 or BRCA1, PALB2$^{CC6/CC6}$ mice are viable, suggesting another loading platform for PALB2 could substitute for BRCA1. Similar to BRCA1/RNF168-deficiency, combining PALB2$^{CC6/CC6}$ homozygosity with RNF168 deficiency was incompatible with viability and PALB2$^{CC6/CC6}$RNF168$^{-/-}$ embryos died before E16.5 (**Fig. 3, Top Right Panel**). Moreover, partial loss of the PALB2/BRCA1 interaction in PALB2$^{+/CC6}$ RNF168$^{-/-}$ cells led to increased PARPi- and cisplatin-induced genomic instability relative to PALB2$^{+/CC6}$, RNF168$^{-/-}$ or PALB2$^{CC6/CC6}$ cells (**Fig. 3, Bottom Panels**).

Thus, when either BRCA1 levels or its interaction with PALB2 is decreased by 50%, cells rely on the RNF168-dependent mode of PALB2 recruitment to sustain HR.

A4. RNF168 does not cooperate with BRCA1 in replication fork protection

Studies have suggested that replication stress response pathways may be partially defective in cells from heterozygous BRCA1 and PALB2 mutation carriers. Moreover, we have shown that the inability to protect stalled replication forks contributes to DNA damage-induced cytotoxicity (**2016 Annual Report**, **Specific Aim 1**). This raises the possibility that PARPi and cisplatin hypersensitivity observed in BRCA1$^{+/\Delta 11}$RNF168$^{-/-}$ cells could result from defects in replication fork protection as well as HR. However, loss of RNF168 did not further increase nucleolytic degradation of replication forks regardless of the kind of BRCA1 mutation (BRCA1$^{+/\Delta 11}$ or BRCA1$^{\Delta 2/\Delta 2}$) (**Fig. 4**).



We conclude that impairment of RAD51-dependent HR, but not replication fork protection, underlies the synthetic lethal interaction between BRCA1$^{+/\Delta 11}$ and RNF168.

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

The project has presented opportunities for the Principal Investigator (**Dr. Andre Nussenzweig**) and a post-doctoral fellow (**Dr. Dali Zong**) to present their work in symposia at the NIH and at national and international conferences (**see details in Section 6 below**)

How were the results disseminated to communities of interest?

Data were shared with the scientific community via informal discussions, posters and presentations at scientific meetings. A manuscript has been recently accepted in *Molecular Cell* and is currently *in press*.

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

Nothing to Report

4. IMPACT: This component is used to describe ways in which the work, findings, and specific products of the project have had an impact during this reporting period. 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:

- the development of the principal discipline(s) of the project;
- other disciplines;
- technology transfer; or
- society beyond science and technology.

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

Work in support of the aims of this proposal highlight multiple mechanisms that can alter drug sensitivity or resistance in BRCA-deficient tumors. In particular, in this annual report we have identified an indispensable role for RNF168 in preventing genome instability resulting from germline heterozygous BRCA1 mutation, the first time a cellular factor has been shown to cause BRCA1 haploinsufficiency. We suggest that deregulation of RNF168 activity could promote cancer predisposition in BRCA1 mutation carriers, and that targeting the chromatin ubiquitin pathway would induce synthetic lethality in a subset of BRCA1-deficient cancers.

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: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:

- Changes in approach and reasons for change.
- Actual or anticipated problems or delays and actions or plans to resolve them.
- Changes that have a significant impact on expenditures.
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Changes in approach and reasons for change

Nothing to Report

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

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

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

Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. Examples of products include:

- publications, conference papers, and presentations;
- website(s) or other Internet site(s);
- technologies or techniques;
- inventions, patent applications, and/or licenses; and
- other products.

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. There is no restriction on the number. However, agencies are interested in only those publications that most reflect the work under this award in the following categories:

Journal publications.

1. ***BRCA1 haploinsufficiency is masked by RNF168-mediated chromatin Ubiquitylation.*** **Molecular Cell**. 2019 (In Press)
Dali Zong, Salomé Adam, Yifan Wang, Hiroyuki Sasanuma, Elsa Callén, Matilde Murga, Amanda Day, Michael J. Kruhlak, Nancy Wong, Meagan Munro, Arnab Ray Chaudhuri, Baktiar Karim, Bing Xia, Shunichi Takeda, Neil Johnson, Daniel Durocher, André Nussenzweig

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

Nothing to Report

Other publications, conference papers, and presentations.

The project has presented numerous opportunities for the Principal Investigator (**Dr. Andre Nussenzweig**) and the post-doctoral fellow (**Dr. Dali Zong**) to present their work in symposia at the NIH and at national and international conferences.

Dali Zong: Post-doctoral fellow

Invited Talks:

1. NCI-Rutgers Mini-Retreat, National Institutes of Health, Bethesda, Maryland, Bethesda, Md, November 2018

Andre Nussenzweig: Principal Investigator

Invited Talks:

1. Invited Speaker, ASH Annual Meeting-Scientific Symposium on Understanding and Modulating the DNA Damage Response, Atlanta, GA, December USA, 2017
2. Invited Speaker, Seminar Boston Children's Hospital, Boston, MA, February 2018
3. Keynote Speaker, Annual Gynecologic Malignancies Retreat Dana-Farber Cancer Institute, Boston, MA, March 2018
4. Invited Speaker, 9th. Journee Roger Monier, Cellular Stress and Cancer, Paris, France, April 2018
5. Invited Speaker, Seminar Princess Margaret Cancer Centre, University Health Network, Toronto, Canada, April 2018
6. Keynote Speaker, Cancer and Aging Meeting, Pittsburgh, PA, June 2018

7. Keynote Speaker, 2018 Mutagenesis Gordon Research Conference, Newry, ME, June 2018
8. Invited Keynote Speaker, Cancer Genome Dynamics Meeting- NYU Perlmutter Cancer Center, New York, September 2018
9. Invited Speaker, Understanding Sequence-Specific Mutations in Cancer, Memorial Sloan Kettering Cancer Center, New York, October 2018
10. Invited Speaker, Oncology Programme of the Institute for Research in Biodedicine (IRB), Barcelona, Spain, October 2018
11. Invited Speaker, workshop on Chromosome Architecture and Topological Stress, Baeza, Spain, October 2018

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

Provide the following information on participants:

- what individuals have worked on the project?
- has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?
- what other organizations have been involved as partners?

What individuals have worked on the project?

1) PI

Name:	Dr. Andre Nussenzweig
Project Role:	PI
Researcher Identifier (e.g., ORCID ID):	N/A
Nearest person month worked:	3

Contribution to Project:	Responsible for the overall direction of the proposed research as well as daily supervision of laboratory activities, personnel, design and interpretation of experiments, preparation of manuscripts and presentations
Funding Support:	Salary supported by the Intramural Research Program (IRP)

2) Key personnel

Name:	Dr. Dali Zong
Project Role:	CRTA
Researcher Identifier (e.g., ORCID ID):	N/A
Nearest person month worked:	12
Contribution to Project:	Responsible for genetic and biochemical assays designed to elucidate the role of RNF168 in homologous recombination. He also made significant contributions to the generation of a mouse model for RNF168 deficiency. Dr. Zong's work, which is related to Specific Aim 1 in this proposal, forms the basis of a manuscript that is in press in *Molecular Cell*.
Funding Support:	Supported by the Intramural Research Program (IRP)

Name:	Dr. Elsa Callen
Project Role:	Staff Scientist
Researcher Identifier (e.g., ORCID ID):	N/A
Nearest person month worked:	4
Contribution to Project:	Responsible for genetic and biochemical assays designed to elucidate the role of RNF168 in homologous recombination. She also made significant contributions to the generation of a mouse model for RNF168 deficiency. Dr. Callen's work, which is related to Specific Aim 1 in this proposal, forms the basis of a manuscript that is in *Molecular Cell* (in press).
Funding Support:	Supported by the Intramural Research Program (IRP)

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?

Nothing to Report

8.SPECIAL REPORTING REQUIREMENTS:

Nothing to Report

9. APPENDICES: