AWARD NUMBER: W81XWH-19-1-0123

TITLE: Stalled Replication Fork Protection Defects as a Predictor of Therapeutic Response

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REPORT DATE: May 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
May 2022	Annual	1MAY2021 - 30APR2022

4. TITLE AND SUBTITLE

Stalled Replication Fork Protection Defects as a Predictor of Therapeutic Response

5a. CONTRACT NUMBER
W81XWH-19-1-0123

5b. GRANT NUMBER
W81XWH-19-1-0123

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
0011294269

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dana-Farber Cancer Institute, Inc.
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8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development Command
504 Scott Street
Fort Detrick, Maryland 21702-5012.

10. SPONSOR/MONITOR'S ACRONYM(S)
AA

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: The major goals of this award are to study prevalence and mechanisms of stalled replication fork protection defects in high grade serous ovarian cancer (HGSC) using patient derived organoid models. The goals of the three Aims included generating and characterizing the organoids, profiling the DNA damage repair capacity of the organoids, and determining if there is synergy between DNA damage repair defect therapies and immune therapies. Progress has been made in all aims this year despite the COVID pandemic induced supply chain issues. Thus far we have generated 34 HGSC organoid cultures and validated them as being matches to their parent tumors. We also profiled the DNA damage repair capacity of these cultures and demonstrated that the majority were proficient in homologous recombination and some deficient in stalled replication fork protection and that these fork protection defects correlated with sensitivity to specific DNA damage repair therapies. We are following all patients from whom organoids are generated and comparing the organoid outcomes with the patient outcomes. In addition, we have tested for activation of the replication stress response in various tumors after single or combination DNA damage repair therapies. We have not identified a common mechanism within ATR signaling which is an overarching signaling pathway in replication stress. We have identified the bromodomain containing protein BRD1 as possibly being important in the replication stress response in HGSC. We continue to work up the mechanism of action of BRD1 and have now found it to be critical in the HGSC replication stress response. It is a relevant therapeutic target. Finally, we immune profiled multiple parent tumors and matched organoid/immune cell co-cultures by flow cytometry and one also by single cell RNA sequencing and demonstrated that the organoid co-cultures are accurate models of the parent tumors. We performed flow cytometry and ELISA functional analyses on these co-cultures and found that immunotherapies do induce an immune response in these cultures and were able to determine that BRD1 depletion by some of these immune therapies is the mechanism by which these therapies bring intra-tumoral immune cells back from exhaustion. We determined that BRD1 inhibitors may be an effective immune therapy in HGSC either alone or in combinations and published these findings in a manuscript submitted with our last report (PMID: 33158814).

15. SUBJECT TERMS
HGSC, DNA damage

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

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

TABLE OF CONTENTS

	Page
1. Introduction	4
2. Keywords	4
3. Accomplishments	4-12
4. Impact	12-13
5. Changes/Problems	13-14
6. Products	15-17
7. Participants & Other Collaborating Organizations	17-19
8. Special Reporting Requirements	19
9. Appendices	19

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

Fifty percent of high grade serous ovarian cancers (HGSC) carry a genomic alteration in a DNA damage repair gene. Through recent functional analysis on a limited number of patient derived HGSC organoids, my lab has demonstrated that the majority of these mutations confer defects in protection/repair of stalled replication forks and not in repair of double strand breaks by homologous recombination as previously thought. Based on this preliminary work we hypothesized that stalled replication fork protection defects are more prevalent than HR defects in HGSC and that therapies targeting such a defect may offer benefit to a larger patient population. The major goal of this work is to use HGSC organoids to understand the importance of fork instability in HGSC, uncover mechanisms leading to fork instability, and determine how such functional defects lead to different types of therapeutic sensitivities, including immune therapies.

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

High grade serous ovarian cancer, DNA damage, stalled replication forks, double strand breaks, homologous recombination, BRCA1, BRCA2, immune therapy, PD-1

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.

-Please note that this award was not started until 11/6/19 due to HRPO approval delays until that date. We experienced significant delays due to a COVID shutdown of Dana-Farber between March and June of 2020 and continued delays in obtaining human tissue for organoid generation continuing through September 2020. Tissue acquisition normalized in the Fall of 2020, and we were able to resume organoid generation successfully at that time. We have requested a second one year NCE to complete the work in our SOW.

Major Task 1: Generation of 100 patient derived human organoid lines from patients on relevant treatments: This task is 40% complete.

Major Task 2: Repair defect characterization of organoids: This task is 40% complete.

Major Task 3: Assess methods of fork destabilization in both fork stable and unstable organoid cultures: This task is 40% complete.

Major Task 4: Assess what other pathways might lead to fork instability in different repair defect backgrounds: This task is 60% complete.

Major Task 5: Immune phenotype parent tumors and organoid cultures in various settings: This task is 80% complete and a publication has been generated (see attached).

Major Task 6: Cytokine profile parent tumors and organoid cultures in various settings This task is 80% complete and a publication has been generated (see attached).

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.

-Please note that this award was not started until 11/6/19 due to HRPO approval delays until that date. We experienced significant delays due to a COVID shutdown of Dana-Farber between March and June of 2020 and continued delays in obtaining human tissue for organoid generation continuing through September 2020. Tissue acquisition normalized in the Fall of 2020, and we were able to resume organoid generation successfully at that time, but we continue to be slowed by supply chain issues, in particular a shortage of basic plastic items and Matrigel. We have requested a second one year NCE to complete the work in our SOW.

Major Task 1: Generation of 100 patient derived human organoid lines from patients on relevant treatments:

1) The major activities that occurred in this task were the generation and characterization of HGSC organoids.

2) Our objective is to generate and profile enough organoids to determine prevalence of stalled replication fork protection defects, correlations with therapeutic response in patients, and correlations with specific therapies or genomic alterations.

3) We were able to generate and histologically profile 14 new HGSC organoid lines since tissue collection resumed in Fall 2020, brining our total to 34. We have been working with the Center for Patient Derived Models at Dana-Farber Cancer Institute to genomically profile the organoids. For each organoid and parent tumor, we are currently performing STR profiling to ensure that they both match each other and are not contaminated with other lines. We are also performing low pass whole genome sequencing to examine copy number alterations and targeted panel whole exome sequencing to search for key somatic alterations (e.g. *BRCA1*). So far, eleven of our organoid lines have completed this sequencing (**Figure 1**), and we will continue genomically profiling all lines generated in the coming year. As a comparison, in Figure 1, we are also showing genomic sequencing of three low grade serous ovarian tumors performed using a separate funding source to show that our HGSC organoid lines are *TP53* mutant as expected and that we can detect relevant mutations in them compared to the low grade subtype. Working with this DFCI internal core facility became our best option due to COVID restrictions. We will continue to generate organoids with all available tissue this coming year. All organoids generated thus far demonstrated p53 mutations, PAX8 positivity, and morphologic characteristics similar to the parent tumors.

4) An additional achievement in this task, is that we have collaborated with Anthony Letai's lab at Dana-Farber in which we are also using BH3 profiling to study the response of organoids generated in Task 1 to drugs. Normally, we use Cell Titer Glo to measure ATP in the cells after dose curve drug treatments to establish sensitivity, and these were the sensitivities we had been using to match to patients. The Letai lab studies apoptosis and uses BH3 profiling to measure the apoptotic response of tumor cells to drugs. With Dr. Letai, we have now, in addition to our own Cell Titer Glo profiling, performed BH3 profiling on 10 of our lines. We are excited to report that the BH3 profiling appears to more accurately reflect patient response than the Cell Titer Glo measurements. We are now preparing these additional studies for publication and hope to submit the manuscript for peer review in the coming year.

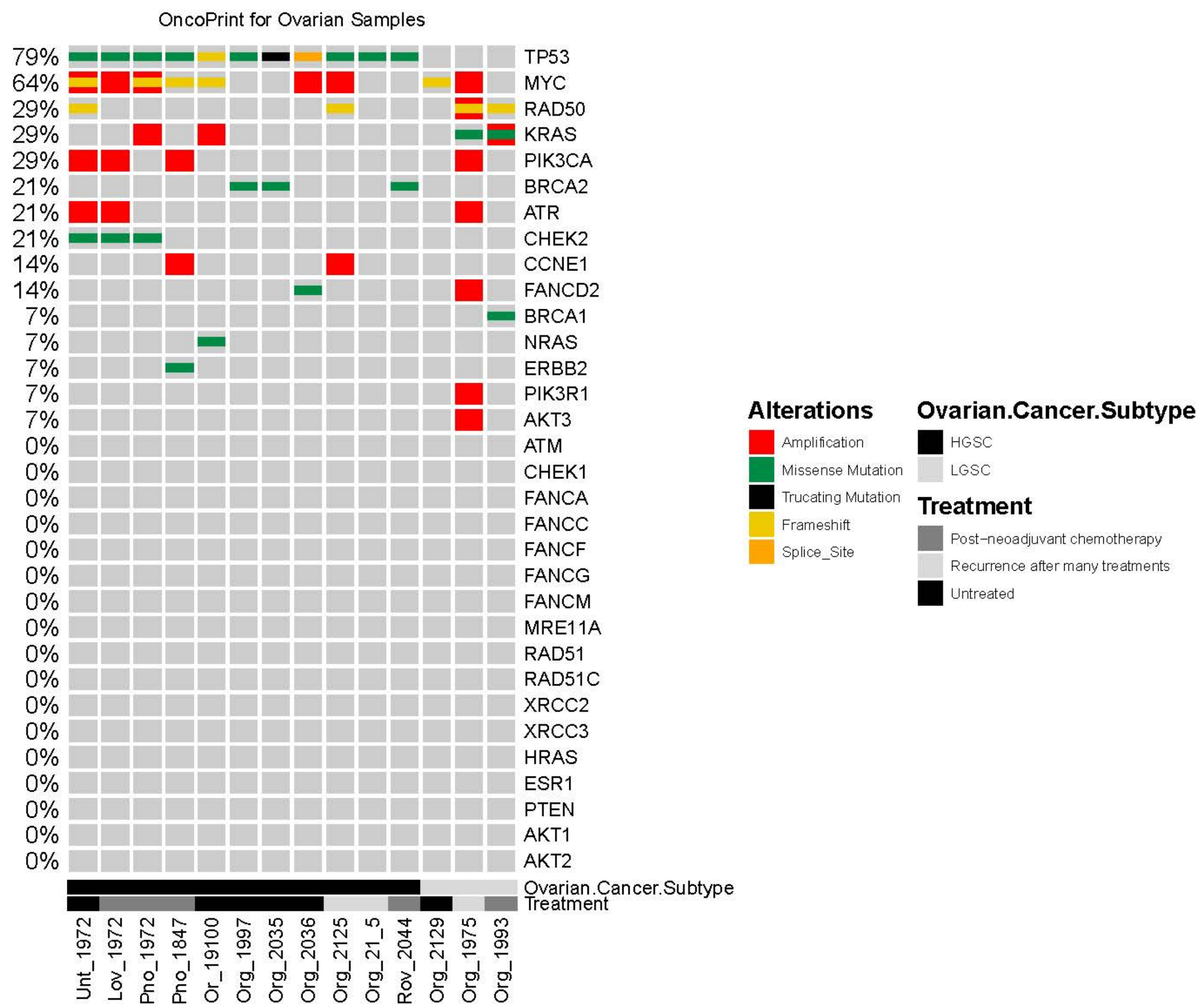

Figure 1: Genomic sequencing of 11 HGSC organoids (left) using this DOD award and three low grade serous organoids on the right (not funded by this award). Relevant genes were assessed for copy number and mutations as shown in the graph with the key to alteration type on the right. The percent of organoids with alterations in each gene is shown on the left of the graph with the gene name on the right. Black and gray boxes demonstrated subtype and treatment status.

Overall, the work in this task is moving forward and provided us with organoid lines to work with in all other Aims.

Task 2: Repair defect characterization of organoids:

1) The major activities in this task are to profile the stalled fork protection capacity, homologous recombination capacity, DNA damage genomic traits, and sensitivity to DNA damage therapies of the organoid lines generated in task 1.

2) The objective is to utilize these findings to compare to patient outcomes which will help determine which, if any, organoid assays may mimic patient response.

3) The major results in this task are that we have profiled the repair capacity of our 34 organoid cultures by performing replication combing assays, RAD51 focus formation assays, and testing the cultures for sensitivity to carboplatin, gemcitabine, and a PARP and ATR inhibitor. Out of the 34 lines none appears to be HR defective, including three known *BRCA1* mutant organoids, but more than half have stalled fork protection defects by the fiber assays and are more sensitive to replication stress inducing agents such as gemcitabine.

4) We continue the work in this aim with every new line generated.

Major Task 3: Assess methods of fork destabilization in both fork stable and unstable organoid cultures:

1) The major activities in this task are to use western blots and replication combing assays to study replication stress in our organoid cultures in the setting of drug combination treatment with the hope that perhaps specific combinations can destabilize replication forks and cause cytotoxicity even in fork stable lines.
2) The objective of this task is to determine if combination therapies may be utilized even in therapy resistant patients by defining combinations which can induce DNA damage repair defects.
3) The major results in this task so far are that in the 34 cultures, it is apparent that classic replication stress markers like phosphorylated RPA or phosphorylated KAP1 are upregulated at different times post-treatment with single or combination replication stress inducing agents. It is not clear yet if the speed of upregulation is dictating therapeutic response.

Major Task 4: Assess what other pathways might lead to fork instability in different repair defect backgrounds
1) The major activities in this task were to explore mechanisms of fork protection defects in different organoid lines through bulk RNA sequencing of select lines after treatment with replication stress inducing agents. The mechanisms are then tested and validated in the organoids.
2) The goal of this task is to understand mechanisms of replication fork protection defects in HGSC, and in so doing, potentially generate better therapies to target the specific defects.
3) In our recent publication, Wan and Hill, *Cancer Research* 2020 PMID: 33158814, we identified a protein called BRD1 as being an important target for immune therapies in HGSC. However, in addition to studying the role of BRD1 in immune cells, we also began to examine its function in HGSC tumor cells. BRD1 is a bromodomain containing protein known to function in epigenetic regulation of many different genes in different cell types. Nothing is known about its role in HGSC tumor cells. As part of a control for our work studying BRD1 inhibition in immune cells, we tested for sensitivity to the BRD1 inhibitor BAY 299 in a small set of HGSC organoids and cell lines compared to T and NK cell lines. We were surprised to find that our *BRCA1* mutant organoid line 17-39 showed the greatest sensitivity to BRD1 inhibition compared to all other cells suggesting possible synthetic lethality between BRD1 inhibition and *BRCA1* functional loss. To validate this possibility we transfected a standard *BRCA1* wildtype HGSC cell line with two different *BRCA1* specific siRNAs and tested the cells for sensitivity to BAY 299 compared to a control transfected line and indeed observed synthetic lethality.

In the past year, we have now determined that BRD1 is an essential gene in HGSC cells, and that it indeed has a direct role in the DNA damage response to stalled replication forks. To do some of the more challenging time course experiments, we have performed the first series of experiments in an HGSC cell line, OVCAR8 and have now been validating our findings in the organoids generated during this award.

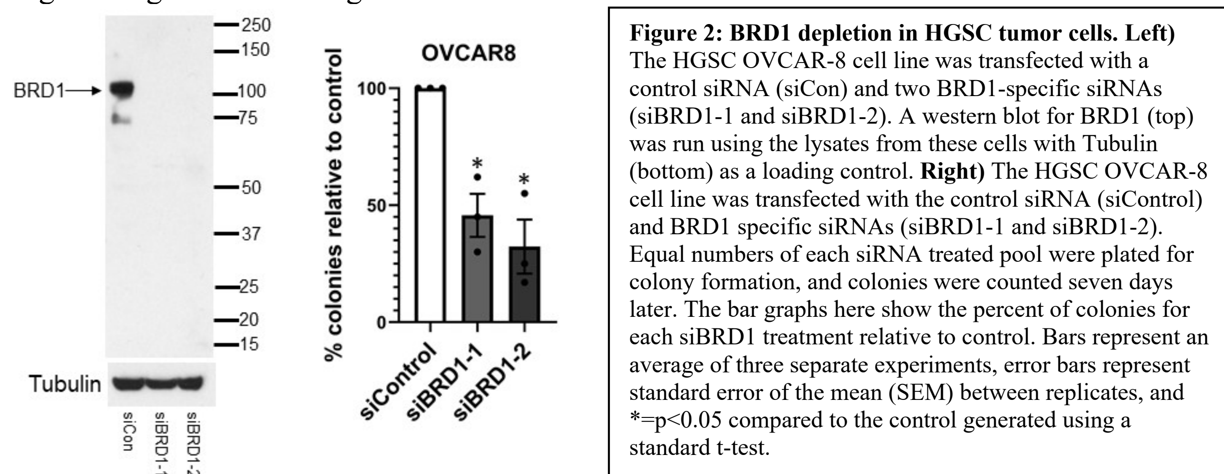

Figure 2: BRD1 depletion in HGSC tumor cells. Left) The HGSC OVCAR-8 cell line was transfected with a control siRNA (siCon) and two BRD1-specific siRNAs (siBRD1-1 and siBRD1-2). A western blot for BRD1 (top) was run using the lysates from these cells with Tubulin (bottom) as a loading control. **Right)** The HGSC OVCAR-8 cell line was transfected with the control siRNA (siControl) and BRD1 specific siRNAs (siBRD1-1 and siBRD1-2). Equal numbers of each siRNA treated pool were plated for colony formation, and colonies were counted seven days later. The bar graphs here show the percent of colonies for each siBRD1 treatment relative to control. Bars represent an average of three separate experiments, error bars represent standard error of the mean (SEM) between replicates, and *=$p<0.05$ compared to the control generated using a standard t-test.

First, we have shown that depleting BRD1 in OVCAR8 cells with gene specific siRNAs compared to a control siRNA causes reduced cell survival, indicating that BRD1 is an essential gene in ovarian cancer cells **(Figure 2)**.

Second, we have shown that BRD1 depletion leads to increased sensitivity to both PARP inhibitors and UV damage, both of which stall replication forks by creating bulky adducts in the DNA which block replication fork progression (**Figure 3**).

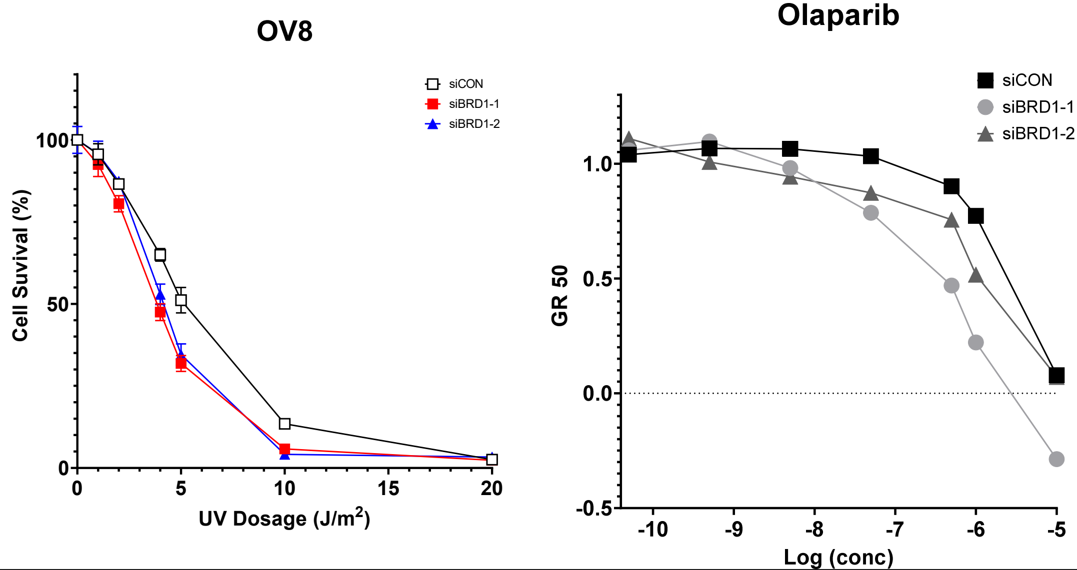

Figure 3: OVCAR8 cells were transfected with either siControl or two BRD1 specific siRNAs and then treated with a dose curve of UVC light (Left) or the PARP inhibitor Olaparib (Right). For UV, survival was measured by colony formation assay, and is shown as percent survival. For Olaparib, survival was measured by Cell Titer Glo and was mathematically growth rate corrected and is shown here as the growth rate corrected IC50 (GR50).

We have also shown that BRD1 localizes to both UV and PARP inhibitor induced DNA damage, meaning that it likely localizes to stalled replication forks physically and is critical for their repair (**Figure 4**).

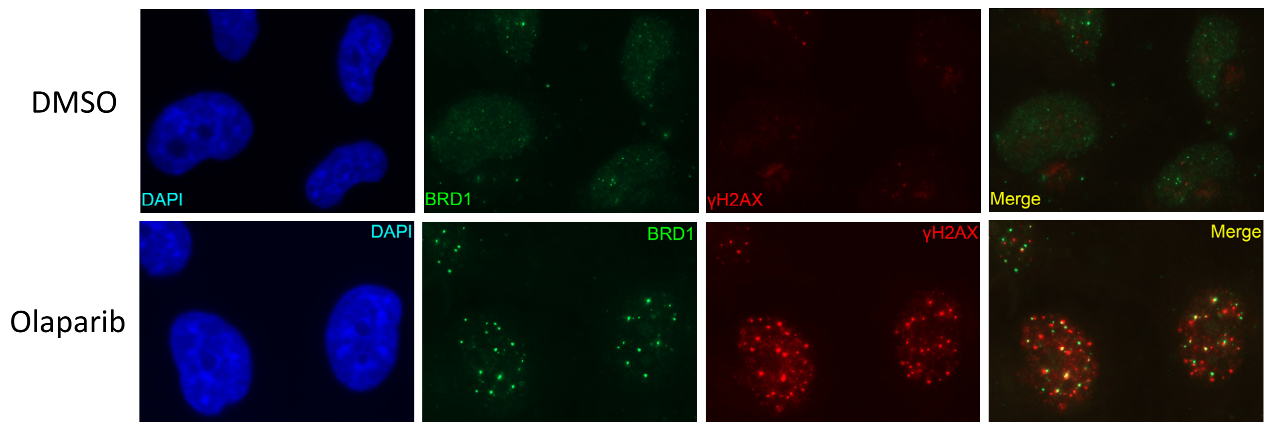

Figure 4: OVCAR8 cells were treated with vehicle DMSO or the PARP inhibitor Olaparib and then stained for BRD1 or the DNA damage marker γH2AX. Shown here are the individual stains and then the merge to demonstrate co-localization of BRD1 and the DNA damage.

Finally, we have shown that there is therapeutic synergy between Olaparib and the BRD1 inhibitor BAY299 in both OVCAR8 cells and in PARP resistant replication fork stable organoids suggesting that BRD1 inhibition may be an effective therapy for HGSC.

We hypothesize that BRD1 has a direct role in the protection or repair of stalled replication forks, and we plan to use the final year on this award to explore this new mechanism of potential replication fork stability/instability in HGSC which was the goal of this award. We hope through this work to define BRD1 inhibition as an effective means of inducing replication fork instability therapeutically in HGSC and thereby causing tumor cell cytotoxicity.

Major Task 5: Immune phenotype parent tumors and organoid cultures in various settings:
1) The major activities in this task were to immune profile parent tumors and treated organoid cultures and have been detailed in our recent publication (PMID: 33158814).
2) The objectives were to be certain that the organoids matched the parent tumors and also to determine if organoid cultures treated with immune therapies alone or in combination with DNA damage repair therapies showed increased tumor cell death with any single agents or combinations.
3) Parent tumors and untreated organoid/immune cell co-cultures were compared to each other by both flow cytometry and single cell RNA sequencing.

We generate these cultures after gentle mechanical disruption of tumors obtained directly from the operating room and then utilize the cultures for various immune functional assays to study anti-tumor immune activity in response to various targeted therapies (PMID: 33158814). In our initial work, we found that the organoid co-cultures contained all of the immune cell types present in the parent tumors at similar ratios by both flow cytometry and single cell RNA sequencing making them faithful models of their parent tumors. In our recent publication we then utilized the co-cultures to study the effects of a novel bispecific anti-PD-1/PD-L1 antibody on all immune cells in the culture in comparison to the single arm anti-PD-1 or anti-PD-L1 controls or an isotype control. There were no obvious changes in cell types or cell numbers in these settings, but we did find increased activity in specific subsets of CD8 T cells and in NK cells in response to the bispecific antibody (PMID: 33158814), and this was a result of decreased expression of the bromodomain containing protein BRD1 induced by the bispecific antibody (PMID: 33158814).

We are now starting to perform flow cytometry profiling on the organoid co-cultures after treatment with the common anti-PD-1 antibody Pembrolizumab or with our BRD1 inhibitor BAY 299 in combination with DNA damage repair agents like carboplatin or gemcitabine. We don't see changes in exhaustion markers when these DNA damage agents are added, however, we do see alterations in our cytokine readouts in task 6. In addition, when we treat the co-cultures with BRD1 inhibitor alone or in combination with PARP inhibition, we see upregulation of NK and T cell activation receptors called NKG2D ligands on the tumor cells in the co-cultures. This suggests that the DNA damage caused by these agents is causing stress on the tumor cells which makes them signal to and activate surrounding immune cells. We will explore this possibility further in the coming year.

4) The results from this initial work were presented at the AACR Annual Meeting in New Orleans this year.

Major Task 6: Cytokine profile parent tumors and organoid cultures in various settings
1) The major activities in this task were to perform cytokine profiling of the parent tumors and organoids.
2) The objective was to demonstrate that the parent tumors and organoids match and that the organoids demonstrate cytokine alterations post treatment with ICB agents alone or in combination with DNA damage repair agents.
3) We started assessing organoid response to ICB agents including a novel bispecific anti-PD-1/PD-L1 antibody on all immune cells in the culture in comparison to the single arm anti-PD-1 or anti-PD-L1 controls or an isotype control using interferon gamma (IFNγ) ELISA after a standard timepoint

Single cell RNA sequencing analysis later revealed that this increased IFNγ production induced by the bispecific antibody was due to downregulation of BRD1 (PMID: 33158814). Thus we tested a BRD1 inhibitor or vehicle in combination with these four antibodies and found that the BRD1 inhibitor increased IFNγ production when combined with isotype control, anti-PD-L1, or

anti-PD-1 suggesting that the BRD1 inhibitor either alone or in combination with ICB agents might be a useful immune therapy in HGSC.

We were able to show by flow cytometry that the BRD1 inhibitor caused decreased exhaustion in T and NK cells to cause this increased IFNγ production (PMID: 33158814). To test whether the BRD1 inhibitor was an effective immune therapy *in vivo*, we studied the efficacy of the BRD1 inhibitor in decreasing tumor burden and increasing immune activity in a syngeneic HGSC mouse model (PMID: 33158814). We found that the BRD1 inhibitor did lead to a significantly decreased tumor burden in these animals and that this was due to decreased T and NK cell exhaustion (PMID: 33158814). These findings validated the efficacy of BRD1 inhibition as an immune therapy in HGSC and also highlighted how effective the co-culture system is in studying the anti-tumor response in HGSC.

We have now performed these same types of analysis with IFNγ and later multi-plex ELISA analysis on organoid co-cultures treated with Pembrolizumab or BRD1 inhibitor combined with DNA damage repair agents. When anti-PD-1 is combined with either carboplatin or the PARP inhibitor Olaparib, we see significant increases in IFNγ production within our co-cultures suggesting that the DNA damage these agents are causing is inducing an increased immune response.

4) An additional achievement based on results from this Aim was that Dr. Hill was awarded an AACR Annual Meeting NextGen Star Award and was able to present these results at the 2022 Meeting in New Orleans.

In the coming year for tasks 5 and 6 together, we hope to understand how the DNA damage is exciting the immune system both through the tumor intrinsic and possibly immune stimulatory mechanisms we have observed.

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.

Training activities for the PI fostered by this award included the opportunity for Dr. Hill who is a junior faculty member to train her new post-doctoral fellow. Being able to train others to perform scientific tasks is critical to success as an independent investigator.

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.

-7-14-20-Dr. Hill presented this work at the Englander Institute for Precision Medicine at Cornell
-10/14/20-Dr. Hill presented this work for the Society for Functional Precision Medicine
-9/23/22-Dr. Hill presented this work at the Dana-Farber Seminars in Oncology
-4-11/22-Dr. Hill presented this work at the AACR Annual Meeting
-5-9-22-Dr. Hill presented this work at the Koch Institute Oncology Seminar Series.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

For Aim 1 (Task 1), we plan to generate and profile the remaining organoid cultures specified in our proposal.

For Aim 2 (Tasks 2-4), we plan to profile the DNA damage repair capacity of the remaining organoids we generate and compare these results to patient outcomes to both determine prevalence of different types of repair defects and understand if any specific organoid assays may help predict patient response. We will also continue to test DNA damage repair agent combinations in our cultures to help determine if specific combinations will be useful in treating patients who may be resistant to DNA damage repair therapies because the tumor lacks a repair defect. Finally, we have now found that BRD1 is an essential gene in ovarian cancer and has a direct role at stalled replication forks. BRD1 is a possible therapeutic target in HGSC. We plan to finish and publish these results on BRD1 in the next year.

For Aim 3 (Tasks 5-6) we have our immune profiling system functioning well and have already published a manuscript with our findings during this past funding year. Our goal in the next year is to test more DNA damage repair agent/ICB combinations, and to understand the mechanism by which our BRD1 inhibitor is reducing T and NK cell exhaustion within the tumor microenvironment.

4. **IMPACT:** D*escribe 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).

The major findings from this work so far have to do with high grade serous ovarian cancer response to immune therapies. Our work in Tasks 5 and 6 allowed us to immune profile multiple ovarian tumors using flow cytometry, ELISA, and transcriptomic assays. This helped us discover that currently available immune therapies like Pembrolizumab do not effectively target specific populations of T and NK cells. By determining this, we have identified BRD1 as an effective immune therapy target in ovarian cancer for which a small molecule therapy does exists. In addition, we have now found that BRD1 inhibitors also have tumor intrinsic effects. Anti-BRD1 therapies may be effective either alone or in combination with current immune therapies in bolstering a successful anti-tumor immune response in HGSC.

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.

Nothing to Report.

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.*

Nothing to Report.

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.*

The work so far this year has had a positive impact on using immune therapy to treat ovarian cancer. Previously these therapies have not been effective in ovarian cancer, but based on work in Aim 3, we have now identified critical cellular and mechanistic targets for immune therapy, specifically the protein BRD1, in ovarian cancer which will help identify currently available therapies for these patients and help guide therapeutic design in the future. In addition, we have now found that BRD1 has tumor intrinsic effects, so therapies targeting BRD1 may both kill tumor cells directly and enhance the anti-tumor immune response.

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.

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.

Supply chain issues, in particular with respect to plastics and Matrigel, presented a major obstacle during this reporting period which caused delays. The COVID 19 pandemic caused a tissue banking ban in late February of 2020 at my hospital which was not lifted until late Summer 2020. In addition, surgical operations did not resume at full capacity until Fall 2020. In addition, my institute was shut down from mid-March until June of 2020. For these reasons, I was not able to collect the necessary tissue to generate new organoid lines until Fall 2020. My team has now been back in the lab full time for more than a year, and we are working hard to complete as much work as we can on Aims 1 and 2 with the organoid lines we generated between 11/19 and 2/20 and now from Fall 2020-April 2022, and we continue to generate new lines with all available tissue. During the shutdown period, Dr. Hill was able to write a manuscript based on the work in this project. We have requested a no cost extension for the coming year to complete the work in our approved SOW.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Please see above. Due to the delayed start due to my human subjects protocol approval, the COVID 19 shutdown, the COVID19 tissue banking issues, and supply chain issues, we are behind on expenditures for this year and have requested an extra one year NCE to complete the proposed work.

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 or care of vertebrate animals

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).*

Wan, C., Keany, M., Dong, H., Al-Alem, L.F., Pandya, U., Lazo, S., Boehnke, K., Lynch, K.N., Xu, R., Zarrella, D.T., Gu, S., Cejas, P., Lim, K., Long, H., Elias, K., Horowitz, N., Feltmate, C.M., Muto, M.G., Worley, M., Berkowitz, R.S., Matulonis, U.A., Nucci, M.R., Crum, C.P., Rueda, B.R., Brown, M., Liu, X.S., **Hill, S.J.** Enhanced efficacy of simultaneous PD-1 and PD-L1 immune checkpoint blockade in high grade serous ovarian cancer. *Cancer Research*. 2021 Jan 1;81(1):158-173. doi: 10.1158/0008-5472.CAN-20-1674. Epub 2020 Nov 6. Yes.

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.

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.*

-7-14-20-Dr. Hill presented this work at the Englander Institute for Precision Medicine at Cornell.
- 10/14/20-Dr. Hill presented this work for the Society for Functional Precision Medicine
-04/2021-Dr. Hill was awarded the *Cancer Research* Early Career Award for the above publication funded by this DOD award (https://cancerres.aacrjournals.org/award).
-04/2022-Dr. Hill received an AACR NextGen Star Award at the 2022 AACR Annual meeting (https://www.aacr.org/meeting/aacr-annual-meeting-2022/nextgen-stars/)

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

  None

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

  None

- **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

- **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;*

Tina's Wish Rising Star Grant-Dr. Hill applied for this award in March of 2019 and received the award in September of 2019.
NIH 1DP5OD029637-01- Dr. Hill applied for this award in September 2019 and received the award in September 2020.
OCRA Ann and Sol Schreiber Mentored Investigator Award -Dr. Hill's postdoctoral fellow received this fellowship in March 2022.

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".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Sarah Hill

Project Role: PI

Research Identifier: ORCID ID 0000-0002-9199-9459

Nearest person month worked: 3

Contribution to project: Dr. Hill has designed and performed all experiments outlined in the proposal, analyzed all data, and written and submitted a manuscript.

Funding Support: This award, a Tina's Wish Rising Star Grant, previously an AACR AstraZeneca Ovarian Cancer Research Fellowship, previously 1K08CA241093-01A1, and now 1DP5OD029637-01.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

There have been no changes during the last reporting period.

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.*

None.

8. SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS: *N/A*

9. QUAD CHARTS: *N/A*

10. APPENDICES: *N/A*