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TITLE: Stalled replication fork protection defects as a predictor of therapeutic response

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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 in the short time since the award began. Thus far we have generated twelve 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 but 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. However, the speed of progression of tumor cells through the cell cycle may dictate response to DNA damage therapies. In addition, after performing bulk RNA sequencing analysis on a subset of these organoids after treatment with replication stress inducing agents, we have identified the protein IKZF3 as possibly being important in the replication stress response in HGSC. We continue to work up the mechanism of action of IKZF3 and other hits from our bulk RNA sequencing in the HGSC replication stress response. Finally, we immune profiled 10 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 immune therapies like Pembrolizumab do induce an immune response but are less effective because they fail to engage specific subsets of T and NK cells. We plan to continue work on all Aims in the coming year.

15. SUBJECT TERMS

NONE LISTED

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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.
Major Task 1: Generation of 100 patient derived human organoid lines from patients on relevant treatments: This task is 12% complete.
Major Task 2: Repair defect characterization of organoids: This task is 12% complete.
Major Task 3: Assess methods of fork destabilization in both fork stable and unstable organoid cultures: This task is 12% complete.
Major Task 4: Assess what other pathways might lead to fork instability in different repair defect backgrounds: This task is 50% complete.
Major Task 5: Immune phenotype parent tumors and organoid cultures in various settings: This task is 50% complete and a publication has been submitted.
Major Task 6: Cytokine profile parent tumors and organoid cultures in various settings This task is 50% complete and a publication has been submitted.

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.

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 12 new HGSC organoid lines for use in all Aims of this project and are on track to reach our goal number by the end of the project. All organoids demonstrated p53 mutations, PAX8 positivity, and morphologic characteristics similar to the parent tumors.

4) One extra achievement in this funding period is that we were able to generate organoids from two patients pre- and post-chemotherapy allowing us to study the changes to the disease through chemotherapy.

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

Major 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 12 organoid cultures by performing replication combing assays, RAD51 focus formation assays, and testing the cultures for sensitivity to carboplatin, gemcitabine, and a PARP, CHK1, and ATR inhibitor. Out of the 12 lines none appears to be HR defective, 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 or an ATR inhibitor.

4) An extra achievement was profiling the pre- and post- treatment organoid cultures. Both cultures remained fork unstable, but the post-treatment cultures showed increased IC50s for platinum, and we will attempt to further dissect the changes in the post-treatment organoids to determine what led to this increased IC50.

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 12 cultures, the cell cycle and replication progression speed have an impact on response to single or combination replication stress inducing agents. By western blot, 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. The speed of upregulation of these markers is now being correlated with apoptosis and cell cycle markers to help determine if the speed correlates with cytotoxicity and if the cell cycle speed dictates response. The speed of replication stress marker upregulation has not correlated with fork instability. Overall, these findings suggest that the cycling abilities of the tumor cells may be a mechanism of response to therapy which we will further investigate.

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) Two of the organoid lines generated thus far were treated with control, gemcitabine, CHK1 inhibitor, or gemcitabine+CHK1 inhibitor and RNA was generated post-treatment and analyzed by bulk RNA sequencing analysis. These data are very recent, and we are still exploring the hits.

Figure 1

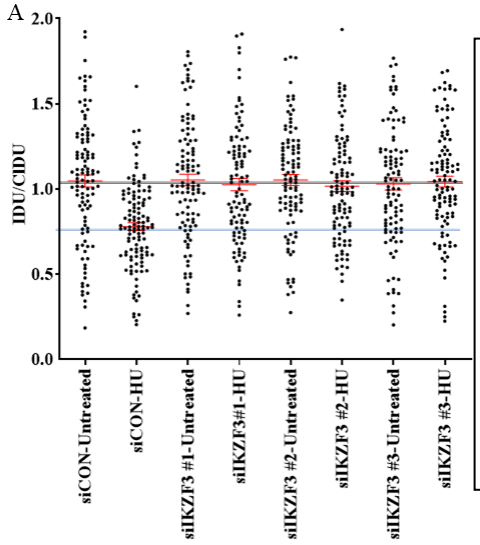

Figure 1. Replication fork stabilization in an ovarian cancer cell line after IKZF3 depletion. DNA fiber assay results from an ovarian cancer cell line, OV8, which has baseline unstable forks after depletion of IKZF3. Cells were transfected with either siControl (CON) or one of three gene specific siRNAs, and 48 hours later the transfected cells underwent DNA fiber analysis. For the fiber assay, cells were sequentially pulsed for equal time periods with two nucleotide analogues, CldU and IdU, followed by exposure to the fork stalling agent hydroxyurea (HU). If the ratio of the track lengths is near one, then the fork was protected during replication stalling. If the ratio of the track lengths is less than one, then the fiber containing the second analogue was degraded, indicating that the tumor cell is unable to protect its stalled forks. The ratio of IdU to CldU is shown for the siRNA transfected cells either untreated or treated with HU. A grey line marks the stable ratio, and a blue line marks the average ratio for the baseline instability of this line seen in the siCON lanes.

However, one hit of interest is a protein called IKZF3. IKZF3 was downregulated after replication stress induction in a post-neoadjuvant chemotherapy fork stable organoid line. IKZF3 has no known role in the DNA damage response and is a lymphoid specific transcription factor. Multiple groups have studied aberrant IKZF3 expression in lung cancer and found that it leads to a more metastatic phenotype in these tumors through down-regulation of expression of proteins involved in cell-cell or cell-matrix interactions and increased epithelial mesenchymal transition (EMT). Based on our findings suggesting potential roles for IKZF3 in the DNA damage response in ovarian cancer, we began to explore potential roles for IKZF3 in the replication stress response.

We utilized a fork unstable HGSC cell line aberrantly expressing IKZF3 (data not shown) to perform initial analysis of the role of IKZF3 in fork stability. In this cell line, treatment with the fork destabilizing agent hydroxyurea causes replication fork instability as measured by the DNA fiber assay indicating a fork protection defect at baseline (Figure 1). In the setting of acute depletion of IKZF3 in this cell line using gene specific siRNAs, the replication forks were stabilized in the setting of hydroxyurea treatment (Figure 1). This depletion also led to resistance to the fork stalling agent gemcitabine (data not shown). This suggests that either IKZF3 localizes to stalled replication forks in the setting of replication stress to perform a protective function or more likely is responsible for a specific transcriptional profile that helps maintain viability of tumor cells with unstable replication forks.

Based on these combined findings, *we hypothesize* that the ability of a HGSC cell to protect stalled replication forks is critical to the overall state of the cell. Tumor cells with initial stalled fork protection defects may adopt specific cellular states driven by certain transcription factors, such as IKZF3 in order to allow for their survival. When a fork unstable tumor experiences selective pressure through chemotherapy treatment, loss of expression of IKZF3 leads to fork stabilization and ultimately survival through therapeutic resistance. It is likely that this resistance mechanism changes the overall properties of the tumor cell both at the molecular level of the replication fork and at the overall transcriptional profile of the cell, which has been observed by others. We are now exploring this hypothesis and also studying other hits.

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.
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 (Figure 2).

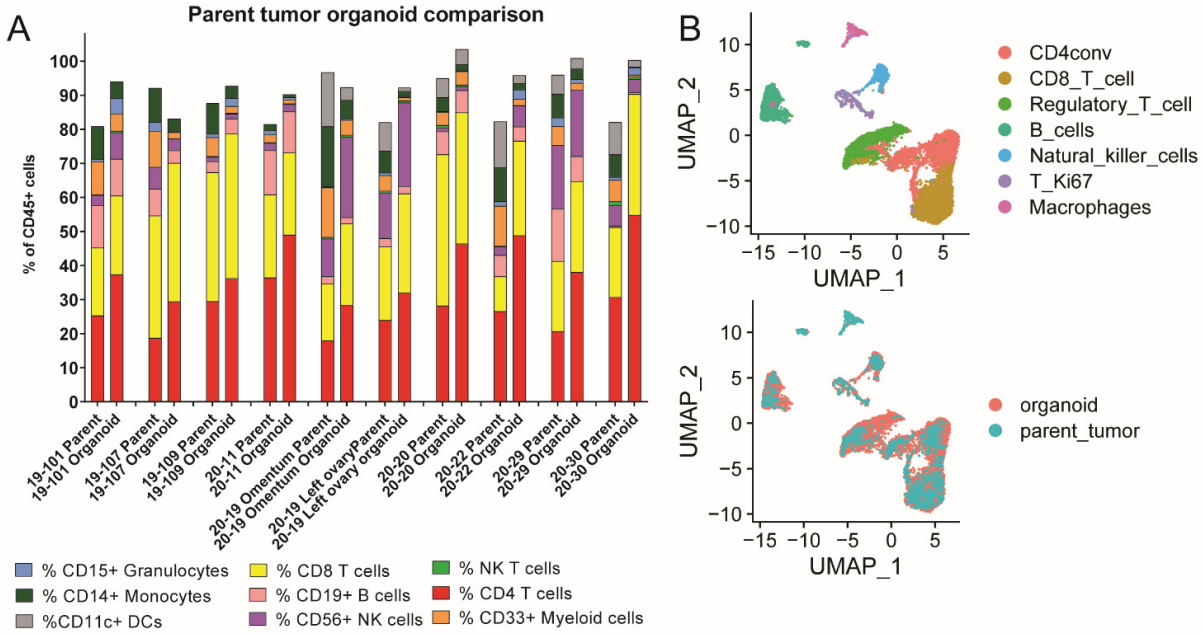

FIGURE 2: Organoid co-cultures resemble parent tumor immune composition: A) Ten short term organoid co-cultures were established, and flow cytometry analysis for all immune lineages of the parent tumor compared to the organoid co-culture was performed at 96 hours post-plating. Flow for dendritic cells was not performed for the first four tumors in the graph. The parent tumor and organoid composition are shown side by side here for each tumor as a percentage of CD45+ cells. **B)** Single cell RNA sequencing analysis was performed on all viable CD45 positive cells from a single parent tumor and organoid co-culture. The top panel shows all cells detected across both samples, and the bottom panel shows an overlay of all cells detected in the parent tumor and organoid co-culture. CD4conv=Conventional non-regulatory CD4 T cells, T_Ki67=proliferating CD8 T cells, Regulatory_T_cell=CD4 Regulatory T cells

To generate organoid co-cultures, my lab obtained HGSCs directly from the operating room, mechanically dissociated them to either generate an organoid line (Major task 1) or a co-culture, and plated the co-cultures in media containing immune checkpoint blockade (ICB) agents or controls. We then performed a series of ELISA and flow cytometry based assays to assess functional response of the immune cells to ICB agents. Flow cytometry analysis comparing immune composition of parent tumors vs. untreated organoid co-cultures from ten patients (Figure 2A) demonstrates that the organoid co-cultures contain all immune cell lineages present in the parent tumor with a slight decrease in macrophages. Single cell RNA sequencing analysis of viable CD45+ cells from a single parent tumor and matched organoid co-culture was also performed (Figure 2B). The single cell sequencing demonstrates similar annotation of all immune lineages in the organoid co-culture compared to the parent tumor showing the same macrophage decrease observed by flow cytometry (Figure 2B). Overall, the co-cultures are faithful models of the parent tumors and do not demonstrate significant alterations from the parent tumors even through treatment.

Profiling the cultures after treatment with the ICB agent Pembrolizumab (anti-PD-1) compared to isotype control demonstrated an increase in Ki67-IFNγ double positive cells. We are now starting to perform flow cytometry profiling on the organoid co-cultures after treatment with pembrolizumab in combination wiht DNA damage repair agents like carboplatin or gemcitabine.

4)The results from this initial work just assessing ICB agent affects in the organoid cultures in these first few months were ultimately so promising that they have been compiled into a manuscript which is now under review. We have just now begun to assess the response of the co-cultures to combinations of ICB agents and DDR agents and do not see significant drops in any immune cell lineages through treatment.

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 using interferon gamma (IFNγ) ELISA after a standard timepoint. We initially assessed Pembrolizumab (anti-PD-1) which is a common and commercially available ICB agent. We were able to demonstrate that in all of our organoid cultures treated with Pembrolizumab, there was increased IFNγ production compared to the isotype control (Figure 3).

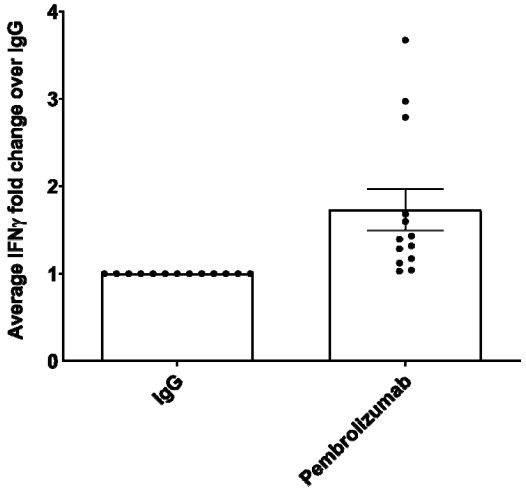

Figure 3: Organoid co-cultures demonstrate an immune response when treated with PD-1 blockade: The organoid cultures profiled in Figure 2 were treated with either an IgG control or Pembrolizumab. Media was harvested after a standard amount of time for each culture, and the media was subject to IFNγ ELISA. Each sample was run in triplicate. The Pembrolizumab result was standardized to IgG control for each tumor, and the result shown here demonstrates the average fold change of the IFNγ over the IgG control for the Pembrolizumab treatment.

Knowing that we could detect an immune response by ELISA in our organoid co-cultures, we have now started testing them with combinations of DNA damage repair defect targeting agents and Pembrolizumab. We initially started with Carboplatin before the COVID-19 shutdown and were able to see a small increase over Pembrolizumab alone (Figure 4).

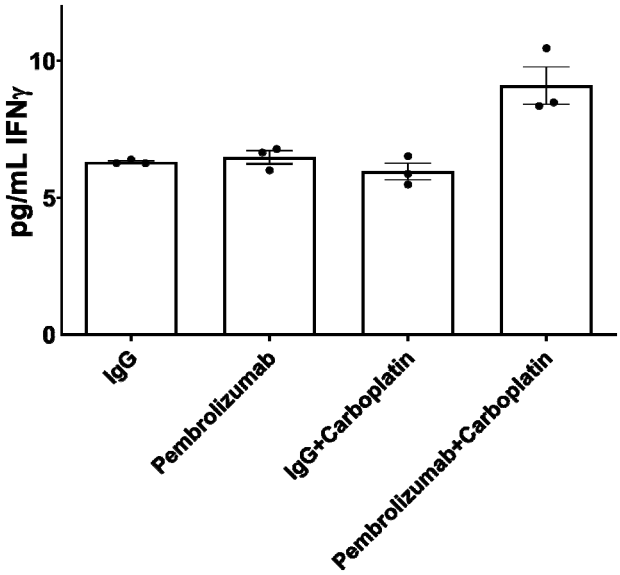

Figure 4: Organoid co-cultures demonstrate an increased response to combinations of pembrolizumab with DNA damage repair agents: An organoid co-culture was treated with either IgG control or Pembrolizumab alone or in combination with a standard dose of carboplatin. Media was harvested after a standard amount of time, and the media was subject to IFNγ ELISA. Each sample was run in triplicate. The pg/mL of IFNγ for each treatment is shown here.

We hope to perform these same types of analysis with IFNγ and later multi-plex ELISA analysis on organoid co-cultures treated with Pembrolizumab combined with other DNA damage repair agents as soon as we are able to collect tissue again.

4) An additional achievement based on results from this Aim is a manuscript submitted detailing our flow cytometry and ELISA profiling of organoid co-cultures post-treatment with ICB agents.

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 research technician. Being able to train others to perform scientific tasks is critical to success as an independent investigator. In addition, Dr. Hill attended the AACR Advances in Ovarian Cancer Research Conference in September 2019 and gave a talk which provided her both educational experience and public speaking experience.

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.

Dr. Hill gave a talk at the AACR Advances in Ovarian Cancer Research Conference in September 2019 on her preliminary data.

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. We hope to begin generating organoids as soon as the COVID19 tissue banking ban at our hospital is lifted.

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 plan to study the mechanisms of replication fork protection defects by further studying IKZF3 and other proteins discovered to be important in this pathway from our RNA sequencing analysis.

For Aim 3 (Tasks 5-6) we have all of our immune profiling system functioning well and have already submitted a manuscript with our findings. Our goal in the next year is to test more DNA damage repair agent/ICB combinations to determine which combinations are most effective in organoids which have DNA damage repair defects.

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 helped identify key cellular and mechanistic targets of effective immune therapies in ovarian cancer which will help identify currently available therapies for these patients and also help to design better immune therapies for these patients in the future.

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 in ovarian cancer which will help identify currently available therapies for these patients and help guide therapeutic design in the future.

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.

Two issues occurred during this reporting period which caused delays. First, HRPO did not approve my human subjects protocol until 11/6/19, and I was not allowed to start the work until that time. Second, the COVID 19 pandemic caused a tissue banking ban in late February at my hospital which has not been lifted yet, and my institute was shut down from mid-March until June of 2020. I am not able to collect the necessary tissue to generate new organoid lines until this ban is lifted. My team is now back in the lab, 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 we will resume organoid line generation as soon as we are able to harvest tissue. Significant progress has already been made on Aim 3, and we will also resume work on that Aim as soon as the ban is lifted. We anticipate requesting a no cost extension at the end of the award because of these delays which was suggested by my program officer.

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, and the COVID19 tissue banking ban, we are behind on expenditures for this year and plan to ask for a no cost extension at the end of the award 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., Lazo, S., Boehnke, K., Gu, S., Elias, K., Horowitz, N., Feltmate, C.M., Muto, M.G., Worley, M., Berkowitz, R.S., Matulonis, U.A., Nucci, M.R., Crum, C.P., 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. (2020) Under Review; 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.*

AACR Advances in Ovarian Cancer Research; Oral Presentation; September 13-16, 2019; Dissecting mechanisms of replication fork stabilization in patient-derived high grade serous organoid cultures and their impact on therapeutic sensitivity and the immune-tumor interaction

- **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;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

None.

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".

<u>Example:</u>

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, and now 1K08CA241093-01A1.

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.

Prior to the start of this award, Dr. Hill became an independent investigator with her own laboratory space and an Assistant Professor research faculty position at Dana-Farber Cancer Institute. For this reason, she is the sole Key Personnel on this award. Previously her adviser Alan D'Andrea was also listed, but this award transitioned to Dr. Hill alone when she became independent (9/1/20).

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: *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.*

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

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.*