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14. ABSTRACT There is a compelling unmet need for any ovarian cancer therapy effective against chemoresistant recurrences, and gene therapy approaches in cancer have been largely underutilized. Furthermore, gene therapy allows delivery of Mullerian Inhibiting Substance (MIS) at concentrations hitherto unachievable in in vivo models. Thus, AAV9-delivered MIS permits testing in vivo, for the first time, the hypothesis that a fetal inhibitor can be developed as an effective anti-cancer agent, initially against patient-derived xenografts (PDXs) in immunosuppressed mice. We hypothesized that MIS will be an effective and safe therapy for women with recurrent ovarian cancer, which is usually refractory to chemotherapy and most often a death sentence. Our preliminary results indicate that MIS is a potent inhibitor of ovarian cancer in vitro and in vivo; however, only a subset of tumors respond to MIS therapy, and both the mechanism of inhibition and the determinants of response remain poorly understood. The purpose of the project is to test the response to MIS using patient-derived primary ovarian cancer cells, elucidate the mechanism of action using next generation sequencing technologies, identify biomarkers of response, and test the efficacy of MIS gene therapy approach in clinically relevant ovarian cancer models.

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Table of Contents

Page

1. **INTRODUCTION:**

There is a compelling unmet need for any ovarian cancer therapy effective against chemoresistant recurrences, and gene therapy approaches in cancer have been largely underutilized. Furthermore, gene therapy allows delivery of Mullerian Inhibiting Substance (MIS) at concentrations hitherto unachievable in *in vivo* models. Thus, AAV9-delivered MIS permits testing *in vivo*, for the first time, the hypothesis that a fetal inhibitor can be developed as an effective anti-cancer agent, initially against patient-derived xenografts (PDXs) in immunosuppressed mice. We hypothesized that MIS will be an effective and safe therapy for women with recurrent ovarian cancer, which is usually refractory to chemotherapy and most often a death sentence. Our preliminary results indicate that MIS is a potent inhibitor of ovarian cancer *in vitro* and *in vivo;* however, only a subset of tumors respond to MIS therapy, and both the mechanism of inhibition and the determinants of response remain poorly understood. The purpose of the project is to test the response to MIS using patient-derived primary ovarian cancer cells, elucidate the mechanism of action using next generation sequencing technologies, identify biomarkers of response, and test the efficacy of MIS gene therapy approach in clinically relevant ovarian cancer models. The scope of the research includes developing the tools and methods to identify the responsive patient population, which includes using new technologies such as single cell RNA sequencing, and improving current PDX models to better reflect the realities of chemoresistant recurrences (including pre-treatment and debulking surgeries). To do so, we collect and characterize cancer cells found in ascites from patients with recurrent resistant disease, and use them in vitro (as tumor spheroids/organoids) to identify response rate, mechanisms of response, and biomarkers while use them in vivo to create patient derived xenografts (PDXs) as a model reflective of the clinical condition, with the associated goal of validating predictors of response and efficacy.
Gene therapy delivery of MIS with AAV9 viral vectors allows one to envision a single-injection, patient-friendly treatment, in combination with other novel or standard therapies using biomarkers to develop treatment algorithms in a manner consistent with personalized medicine principles to maximize response.

2. **KEYWORDS:**

AMH, MIS, PDX, chemoresistance, gene therapy, AAV9, scRNA-seq, inDROP, DROP-seq, ascites, biomarker, personalized medicine, tumor spheroids, tumor organoids, immune cells, immune-oncology.

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Task1: Test the response of a novel recombinant Mullerian Inhibiting Substance (MIS) analog in primary ovarian cancer cells and elucidate the mechanism of action.
1a- Submit any protocols or amendments to IRB committee. (month1-6)
1b- Collect and bank new patient samples, and derive new cell lines. (months 1-60)
1c- Produce recombinant MIS necessary for planned experiments. (months 1-60)
1d- Characterize drug sensitivity (dose-response) for each cell line using spheroid assays treated with a range of drug concentration (MIS, carboplatinum, doxorubicin) and combinations thereof (isobologram). (months 1-24)
1e- Quantify rate of apopotosis in spheroid culture treated in vitro with MIS. (months 1-24)
1f- Quantify the inhibition of stemness using in vitro assays. (months 1-24)
1g- Validation of biomarkers by qPCR. (months 36-60)
1h- Validation of selected gene targets and their role in growth inhibition in the spheroid assay using lentiviral ORF and shRNA. (months 36-60).
Milestones: *1) To have determined the response rate, and dose sensitivity to MIS of at least 20-30 primary cell lines. 2) To differentiate cytostatic from cytotoxic effect of MIS, either alone or in combination with chemotherapy, particularly on the stem cell compartment. 3) To show modulation of the MIS inhibition of spheroid growth following expression of an ORF or shRNA targeting a candidate gene.*
Anticipated outcome: *Our preliminary data suggests a response rate of about 60% in spheroid growth assay, and that the effect may be cytostatic with MIS inducing upregulation of CDK inhibitors, although this hasn't been tested in spheroids yet. We expect to find new gene targets of MIS responsible for the inhibition, and we can use the MISR2 (ORF, shRNA) as a control in the spheroid assay.*

Task2: Examine the gene expression signature of MIS-response in primary cancer cells and develop predictive biomarkers to identify responsive patients.
2a- Screen for expression of canonical MIS/MISR2 pathway genes. (months 1-24).
2b- Perform RNA-seq on at least 10 new primary cell lines. (months 1-24)
2c- Analyse RNA-seq data to identify novel responsive pathways and biomarkers. (months 24-36)
2d- Validate candidates (see task 1g/1h).
2e- Perform Drop-seq on selected responsive primary lines to identify gene expression changes in stem cells (months 36-48).
Milestones: *1) To have confirmed presence of the genes required for the MIS canonical pathway (such as the receptor in all primary cell lines. 2) To perform RNA-seq on a minimum of 10 (up to 30) primary cell lines treated with MIS. 3) Find new pathways regulated by MIS. 4) Perform Drop-seq on 3 patient lines that are highly responsive to MIS.*

Anticipated outcome: *Our preliminary data suggests that there are new uncharacterized pathways regulated by MIS, and we anticipate some may be unique to responsive patient lines. We hope to perform Drop-seq on at least 3 patients, and get coverage of about 1000 cells per patient, which should be sufficient to detect a gene signature in rare stem cells.*

Task3: Test the efficacy of AAV9-LRMIS gene therapy in an orthotopic patient-derived xenograft (PDX) chemoresistant recurrence model and validate biomarkers of response.
3a- Submit any protocols or amendments to IACUC committee. (months 1-6)
3b- Evaluate model of surgical debulking + chemotherapy using previously characterized PDX model. (months 1-12)
3c- Evaluate inhibition of chemoresistant recurrences using new PDX with known in vitro sensitivity to MIS. (months 12-60) N=300 mice needed.
3d- Evaluate proof of concept of new drug combinations with MIS or gene therapy targets in a limited pilot studies (N=10 mice per target) (months 48-60).

Milestones: *1) To have an optimized protocol of labeling primary cell lines, xenografting IP, following tumor growth, surgical debulking, chemotherapy treatment and gene therapy with AAV9-LRMIS with acceptable implantation rates and treatment survival. 2) To perform the surgical debulking model on at least 10 (up to 30) patient cell lines. 3) To evaluate at least one new drug combination or novel gene therapy target in at least 3 patients.*
Anticipated outcome: *Our preliminary data suggests that the surgical debulking model is feasible and that approximately 60% of the PDXs respond to AAV9-LRMIS in vivo, therefore we expect to see a significant increase in progression-free survival in a similar proportion of patients. We hope to generate proof of concept data of novel small molecule combination or gene therapy biologic targeting synergistic pathways to MIS, and to use this preliminary data to procure additional funding to ensure the continued success of developing gene therapy with MIS, and second generation treatments and to translate these findings to the clinic.*

- **What was accomplished under these goals?**

1) Major activities: During this reporting period we have made additional progress on all 3 tasks and continued to reach new milestones. More patient samples were banked to evaluate in vivo and in vitro, and the cell lines derived from these samples have been further characterized. We have performed bulk RNA-sequencing on 14 primary cell line, and 4 control lines (2 leukemia, and 2 adrenal carcinoma cell lines with known high expression of MISR2), treated with MIS for 24h at 10ug/ml or vehicle control. Putative pathways and target genes downstream of MIS were identified, and their regulation by MIS is being validated by qPCR using tumor spheroid cultures treated with recombinant MIS at 10ug/ml. We have also confirmed bioactivity of MIS in a functional in vitro assay using scratch wound closure, which will help distinguishing the types of antitumor responses of MIS. We have generated MISR2 KO lines to serve as negative controls in such assays.

2) Specific objectives applicable for this reporting period:

Task1:
1b- Collect and bank new patient samples, and derive new cell lines. (months 1-60)
We have banked 6 new patient ascites, from which we working to derive new cell lines to test for tumorigenicity in mice.

1c- Produce recombinant MIS necessary for planned experiments. (months 1-60)
We have produced over 50mgs of recombinant MIS during this period, which was sufficient to perform all the proposed studies.

1d- Characterize drug sensitivity (dose-response) for each cell line using spheroid assays treated with a range of drug concentration (MIS, carboplatinum, doxorubicin) and combinations thereof (isobologram). (months 1-24)
In addition to the dose-response experiments for MIS, caboplatinum, and doxorubicin in 12 primary cell lines evaluated in the last funding period we have evaluated the response to carboplatinum of a patient cell line (ptH) with isogenic clones where MISR2 was deleted using CRISPR/CAS9. Intriguingly we observe increased sensitivity to carboplatinum in the MISR2 KO cells. We are currently evaluating combination therapies in both unmodified cell lines and MISR2 KO lines to serve as controls (such as MIS+carboplatinum). 75% completion.

1e- Quantify rate of apopotosis in spheroid culture treated in vitro with MIS. (months 1-24)
We have assessed apoptosis and viability of cells in spheroid assays with 12 patient cell lines with a dose response of MIS and did not observe significant inhibition at low doses (1-20ug/ml). We do find some inhibition of viability using cell titer glo ™ reagents at high doses (80ug/ml) in a majority of patient lines. 100% completion.

1f- Quantify the inhibition of stemness using in vitro assays. (months 1-24)
Our initial analysis of downstream gene markers of MIS indicated that transition between epithelial and mesenchymal cell states may be affected by MIS, which may be the basis of the inhibition of cancer stem cells. We have identified key genes modulating these characteristics which are upregulated by MIS in a subset of patients such as ALDH1A1, KRT5, RBP2. This has prompted us to investigate cell-based assay that can explore inhibition of epithelial to mesenchymal transition by MIS such as scratch wound assays. 100% completion.

1g- Validation of biomarkers by qPCR. (months 36-60)

We have performed preliminary analysis of a panel of candidate biomarkers identified in 14 patient samples evaluated by bulk RNA-sequencing. We are currently validating those markers by qPCR. 10% completion.

1h- Validation of selected gene targets and their role in growth inhibition in the spheroid assay using lentiviral ORF and shRNA. (months 36-60).
We have not yet evaluated lentiviral ORF or shRNA to candidate mediators of response, however we have begun using CRISPR/CAS9 to knockout MISR2 in a subset of cell lines to serve as controls. Currently ptH MISR2 KO clones have been fully validated and the technique has provided superior results to siRNA, which otherwise has low effectiveness in primary cell lines. We will use this technique to further investigate downstream mechanisms of MIS. 10% completion.

Milestones:
1) To have determined the response rate, and dose sensitivity to MIS of at least 20-30 primary cell lines.
We have already achieved this milestone.
2) To differentiate cytostatic from cytotoxic effect of MIS, either alone or in combination with chemotherapy, particularly on the stem cell compartment.
We have tested the cytostatic and cytotoxic effect of MIS alone (user Cell Titer Glo), performed drug –response curves to MIS, carboplatin and doxorubicin, and have tested the sensitivity of MISR2 KO clones to carboplatin. We are ready to assess drug combinations going forward. We begun validating new biomarkers of response of MIS identified using bulk RNA sequencing of 14 cell lines derived from patient samples treated with MIS which identified key genes important in EMT and stemness.
3) To show modulation of the MIS inhibition of spheroid growth following expression of an ORF or shRNA targeting a candidate gene.
We have not yet validated candidate biomarkers of response by performing loss of function but have validate the use of CRISPR/CAS9 to KO target genes.

Task2:
2a- Screen for expression of canonical MIS/MISR2 pathway genes. (months 1-24).
We have analyzed bulk RNA sequencing of MISR2 and MIS in 14 patient lines and find that expression of MISR2 is modest in patient lines, and made the unexpected discovery that all patient lines express relatively high levels of MIS. Furthermore treatment with exogenous MIS does not further activate canonical downstream pathway genes (ID2, ID3, SMAD6), suggesting novel mechanisms of action. 100% completion.

2b- Perform RNA-seq on at least 10 new primary cell lines. (months 1-24)
We have performed bulk RNA-seq in 14 ovarian cancer patient sample-derived cell lines (AM, BD, AO, BG, AQ, H, AS, AV, D2, W, AY, AK, BI, BN), 2 leukemia cell lines controls (K562, HEL), and 2 adrenal carcinoma line controls (H295R, ACC) treated with MIS (10ug/ml, 24h) or vehicle control.

2c- Analyse RNA-seq data to identify novel responsive pathways and biomarkers. (months 24-36)
In addition to our previous single cell RNAseq experiments in 3 patients we have analyzed bulk RNA seq of 14 patient cell lines treated with MIS. We have identified novel downstream genes which may be regulated by MIS, including HSPA6, HSPA7, SPP1, CXCL14, ALDH1A1, OAS2, OASL, IFIT1, IFIT2, CYP17A1, H19, KRT4, KRT5, KRT13, KRT14, ZEB2, and two regulatory nodes SOX3-NR5A1-LHX9 and KRT5-ALDH1A1-RBP2. We are currently evaluating those markers and correlating with results from assays related to drug response, EMT, viability, and migration. 25% completion.

2d- Validate candidates (see task 1g/1h).
(see task 1g/1h).

2e- Perform Drop-seq on selected responsive primary lines to identify gene expression changes in stem cells (months 36-48).
We have performed inDROP sequencing in 3 patient samples, and have used the 10X genomic platform to evaluate potential autologous mouse model of ovarian cancer developed by a collaborator (Robert Weinberg at the Whitehead institute) which could allow us to investigate conserved mechanisms of action that we identify in human cell lines. 10% completion.

Milestones:
1) To have confirmed presence of the genes required for the MIS canonical pathway (such as the receptor in all primary cell lines).
We have confirmed the presence of low levels of MISR2 in 14 primary cell lines by bulk RNA-seq along with the absence of induction of canonical downstream targets (ID2, ID3, SMAD6).

2) To perform RNA-seq on a minimum of 10 (up to 30) primary cell lines treated with MIS.
We have performed RNA seq in 14 ovarian cancer primary cell lines to date, but have performed these analyses using only one singlet experiment, which may need to be replicated depending on the success of the qPCR validation.

3) Find new pathways regulated by MIS.

We have identified potential new pathways modulated by MIS including genes involved in EMT and stemness such as ALDH1A1 and KRT5/13/14, and ZEB2.

4) Perform Drop-seq on 3 patient lines that are highly responsive to MIS.
We have previously performed inDROP sequencing on 3 patient lines treated with MIS or vehicle control (2500 cells per sample) however validation of genes putatively regulated by MIS has showed inconsistent regulation across patient lines suggesting additional studies may need to be performed once cell lines responsive to MIS have been identified by bulk RNA sequencing.

Task3:
3a- Submit any protocols or amendments to IACUC committee. (months 1-6)
Our IACUC protocols have been updated and are still active. 100% completion.

3b- Evaluate model of surgical debulking + chemotherapy using previously characterized PDX model. (months 1-12)
We have continued to refine our PDX models, recently comparing the rate of growth of tumors of D and H cell lines luciferized and delivered IP in Nu/Nu, R2G2 and NSG mice. We find that luciferized lines can be readily used to monitor tumor development, and that R2G2 and NSG mice have much faster tumor growth rates than Nu/Nu mice.

3c- Evaluate inhibition of chemoresistant recurrences using new PDX with known in vitro sensitivity to MIS. (months 12-60) N=300 mice needed.
We have tested AAV9-MIS (or control) delivered in mice grafted with 5 different cell lines IP and SQ in autologous immune proficient mouse models in a small pilot study (N=3 per group). We have observed both suppression and activation of tumor growth that will be validated in larger group sizes based upon power calculations.

3d- Evaluate proof of concept of new drug combinations with MIS or gene therapy targets in a limited pilot studies (N=10 mice per target) (months 48-60).
We have not yet tested combination therapies with MIS in our PDX models.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

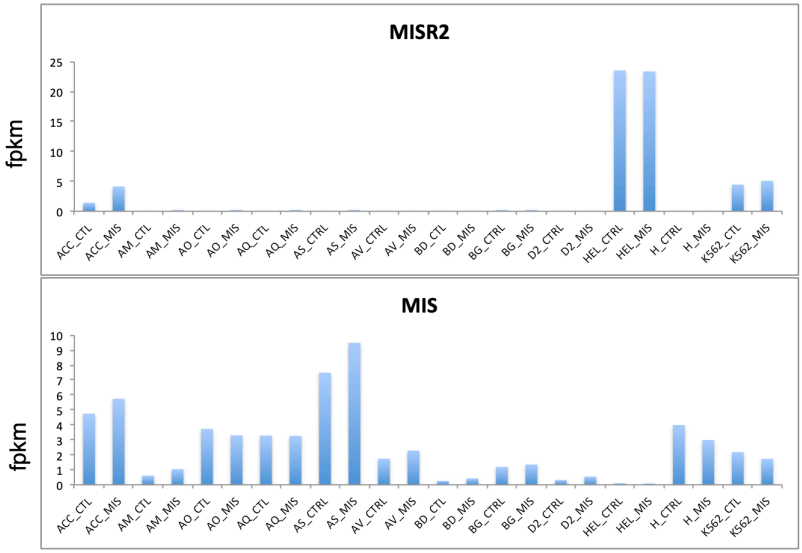

Fig1. Expression of MIS and MISR2 in a panel of primary ovarian cancer cell lines and other cancer controls by RNAseq.

During the reporting period we have made significant progress in understanding the pathways of MIS/MISR2 in ovarian cancer. Importantly we have performed a large scale screening experiment involving the treatment of 14 ovarian cancer patient sample-derived cell lines (AM, BD, AO, BG, AQ, H, AS, AV, D2, W, AY, AK, BI, BN), 2 leukemia cell lines controls (K562, HEL), and 2 adrenal carcinoma line controls (H295R, ACC) treated with MIS (10ug/ml, 24h) or vehicle control. These parameters were chosen following multiple rounds of optimization for dosing and timing of collection using an initial set of candidate genes identified by single cell RNA sequencing using 3 patients, the most striking of which was H19 which was found to be both upregulated or downregulated or not regulated by MIS depending on the patient cell line. The RNA sequencing experiment was very informative, showing us that ovarian cancer has low level of expression of MISR2 compared to our positive control cell lines, ranging in the 0-4 fpkm (Fig1). Surprisingly all ovarian cancer cell lines tested had detectable levels of MIS (Fig1). The classical downstream markers of MIS response that we and others have identified in other cell types (granulosa cells, uterine cells, ovarian surface epithelium) such as ID2, ID3, SMAD6, IGFBP5 and BAMBI were not modulated in the ovarian cancer cell lines nor the control other cancer cells (Fig2). However new genes were found to be consistently upregulated in multiple cell lines such as SPP1 (K562, H, AO), or ALDH1A1 (H, D2, AO), and others (Fig2). We have begun validating the regulation of these genes by MIS using qPCR in large triplicate experiments across the 14 primary cell lines and controls. Intriguingly some networks were found to be consistently perturbed by MIS in multiple cell lines such as KRT5-ALDH1A1-RBP2 (Fig3) which may help explain our observation of inhibition of the stem cell compartment and our new findings of dysregulation of EMT by MIS. To explore these mechanisms we are screening the patient lines grown as monolayers (for those where this is possible) using scratch wound assays. We have found for example significant inhibition of cell motility and migration in H cells by MIS at doses of 10ug/ml and 100ug/ml but not 1ug/ml or vehicle control. Furthermore KO of MISR2 in H cells resulted in significant sensitization of those cells to carboplatinum, suggesting that despite the low expression levels of MISR2, these pathways are both active and responsive to MIS, and significantly contribute to tumorigenicity and chemoresistance.

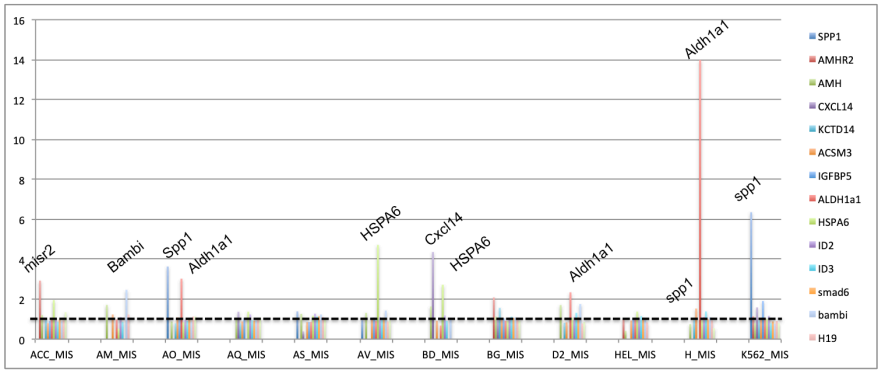

Fig2. A panel of primary ovarian cancer cell lines and control cells showing fold induction of candidate biomarker genes with MIS treatment by RNAseq.

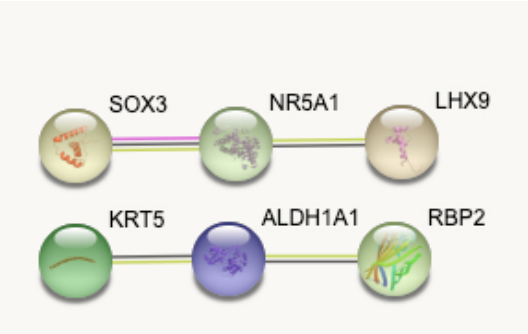

Fig3. Two regulatory nodes with consistent regulation by MIS across several patients by RNAseq.

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

I participated in weekly laboratory floor meetings, where both students and principal investigators present their work, where I was able to receive feedback from my peers about the ongoing progress of my project. I also benefit from attending weekly seminars on cancer from the MGH Cancer Center seminars that were directly relevant to my project. My team also presented three posters at the Dana-Farber Harvard Cancer Center Gynecological Cancer Research Day, which was a great opportunity to show my work and get critical feedback on ideas and projects from my peers and experts in ovarian cancers.

I had the opportunity to attend grant-writing classes (R01), which will be critical in securing continued financing of my ovarian cancer projects. I also participated in faculty meetings by the Executive Committee on Research, which allowed me to be exposed to issues affecting the research faculty, including funding opportunities and challenges and resources available to young investigators. I serve as a representative of the Surgery Department on the Committee on Fundamental Research of MGH which has allowed to be involved in the decision making process to facilitate research in my institution.

I presented some of my ovarian cancer work at the Marsha Rivkin/AACR meetings in September 2018 following the Ovarian Cancer Academy meeting. I enjoyed greatly meeting with the other awardees, mentors, and the Deans. I have developed a collaboration with another academy member, Anirban Mitra, which I hope to continue to go forward. I have talked to many other PIs about establishing new collaborations. I have also forged a new collaboration with Robert Weinberg at the Whitehead Institute which has allowed me to gain access to autologous mouse models of ovarian cancer, which will enable me to assess the effect of MIs not only on the cancer cell, but also on the tumor microenvironment and immune cells.

I have been able to meet with Chief of the Department of Pediatric Surgery, Allan Goldstein, regarding my career progress, where he helped me identify short term goals for my promotion to associate professor. I have also established a new working relationship with the new director of Gynecologic Oncology, David Sprigs who has offered to replace Michael Birrer as my "other mentor" pending approval by the DOD ovarian cancer academy. I continue to meet weekly with Dr. Donahoe who is mentoring me on managing a laboratory, crafting compelling grant applications, negotiating institutional support, establishing collaborations, and helping me further my career goals. I have recently submitted a new R01 which scored in the 15th percentile with an impact score of 27 which has the potential to be funded.

I have continued my work on a team grant to the OCRA with David Sabatini and Jeremiah Johnson looking at ovarian cancer susceptibilities with CRIPS/CAS9 screens by leveraging the patient samples and cell lines, which I continue to collect thanks to this DoD grant.

I expect to submit a manuscript on MIS in ovarian cancer by the end of the year.

Conferences:

David Pépin, Motohiro Kano, Hatice Saatcioglu, Jennifer Hsu, LiHua Zhang, Nicholas Nagykery, Raghav Mohan, Rana Suliman, Dan Wang, Guangping Gao, Mary E Sabatini, Patricia K. Donahoe. Müllerian Inhibiting Substance Control of Primordial Follicles. Endocrine Society meeting. March 17-20 2018, Chicago, IL. (invited platform presentation).

David Pepin, Motohiro Kano, Amanda Sosulski, Li Hua Zhang, Dan Wang, Guangping Gao, Patricia K Donahoe. Mechanisms of Protection of the Ovarian Reserve by MIS During Chemotherapy. Meeting of the Society for the Study of Reproduction. July 10-13th 2018, New-Orleans, LA. (invited platform presentation).

David Pépin, Lindsey Vansandt, Amy Miller, Jackie Newsom, Motohiro Kano, Lihua Zhang, Nicholas Nagykery, Dan Wang, Guangping Gao, Patricia K. Donahoe, William Swanson. Gene Therapy with AAV9 Delivery of an MIS Transgene Inhibits Estrus in Female Cats. ACC&D 6th International Symposium on Non-Surgical Methods of Pet Population Control. July 22-24 2018. Boston, MA. (platform presentation)

Raghav Mohan, Motohiro Kano, Hatice Saatcioglu, Nobuhiro Takahashi, LiHua Zhang, Nicholas Nagykery, Joy Poulo, Patricia K Donahoe, **David Pepin**. A single cell RNA-sequencing approach to uncovering human ovarian tumor and immune cell heterogeneity, and their response to Mullerian Inhibiting Substance using patient ascites samples. The Rivkin Center for Ovarian Cancer & the American Association for Cancer Research (AACR) 12th Biennial Ovarian Cancer Research Symposium. September 13-15th, 2018. Seattle, Washington.

- **How were the results disseminated to communities of interest?**

I am hosting two returning high-school students (Selena Wu, Augustin Wright), and an honour student (Betsy Preciozo) in the laboratory this summer who are working on the ovarian cancer projects which is important to expose young scientists to careers in the field of ovarian cancer research. I have also submitted an abstract to the upcoming ovarian cancer

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

Regarding task 1, to test the response of a novel recombinant Mullerian Inhibiting Substance (MIS) analog in primary ovarian cancer cells and elucidate the mechanism of action. We will perform additional relevant cell based assays such as scratch wound. We will also compare those mechanisms with experiments performed on mouse ovarian cancer cell lines which have robust expression of the receptor to find commonalities.

Regarding task 2, to examine the gene expression signature of MIS-response in primary cancer cells and develop predictive biomarkers to identify responsive patients. We will complete the analysis and validation of the panel of 14 primary ovarian cancer cell lines. We may perform replicate experiments to sequence or add additional cell lines to increase robustness of findings.

Regarding task 3, to test the efficacy of AAV9-LRMIS gene therapy in an orthotopic patient-derived xenograft (PDX) chemoresistant recurrence model and validate biomarkers of response. We will continue to evaluate gene therapy using both cell lines which appear to be responsive kin vitro with our newly identified biomarkers and murine mouse models of ovarian cancer, in which we can track the involvement of the immune response in MIS inhibition of tumor growth.

Finally, we plan to submit a manuscript by the end of the next reporting period, regarding our findings on the response rate of primary ascites cultures and the mechanisms of action of MIS assessed by RNA-sequencing.

4. **IMPACT:**

Two techniques developed for this project are likely to make an important impact in the field of ovarian cancer:

In collaboration with Robert Weinberg, we have developed a new immune competent mouse model of ovarian cancer, which will allow us to validate mechanisms of action of MIS involving the immune system and the tumor microenvironment. These models are poised to greatly benefit the ovarian cancer research community who desperately needs more immune competent models.

Secondly, we have developed a large number of patient cell lines and PDX models that are a useful resource to the Ovarian Cancer Academy awardees and the research community at large to test hypothesis in a patient-relevant models.

- **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:**

We are evaluating changing the "other mentor" from Michael Birrer to David Spriggs.

6. **PRODUCTS:**

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

12th Biennial Ovarian Cancer Research Symposium (Rivkin Center for Ovarian Cancer and the American Association for Cancer Research), September 13th-15th, 2018. A single cell RNA-sequencing approach to uncovering human ovarian tumor and immune cell heterogeneity, and their response to Mullerian Inhibiting Substance using patient ascites samples. Raghav Mohan, Motohiro Kano, Hatice Saatcioglu, Nobuhiro Takahashi, LiHua Zhang, Nicholas Nagykery, Joy Poulo, Patricia K Donahoe, David Pepin.

- **Journal publications.**

Nothing to report.

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

Nothing to report.

- **Other publications, conference papers, and presentations.**

Whiting G, Ferguson J, Fang M, Pepin D, Donahoe P, Matejtschuk P, Burns C, Wheeler JX. Quantification of Müllerian Inhibiting Substance/Anti-Müllerian Hormone polypeptide by isotope dilution mass spectrometry. Anal Biochem. 2018 Nov 1;560:50-55. doi: 10.1016/j.ab.2018.05.006. Epub 2018 May 6. PubMed PMID: 29742446.

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- **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**

  - *biospecimen collections:*

We banked 6 new patient ascites, from which we are attempting to derive cell lines, and PDX models.

  - *models;*

We have developed an autologous mouse model of ovarian cancer in collaboration with Robert Weinberg which we are currently characterizing.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project:**

Name:	*David Pepin*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0003-2046-6708
Nearest person month worked:	*4*
Contribution to Project:	*Data analysis and coordination of the project.*
Funding Support:	

Name:	*Motohiro Kano*
Project Role:	*Fellow*
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*2*
Contribution to Project:	*Kano performed xenografts and surgeries on model. Established primary cell lines*
Funding Support:	

Name:	*Lihua Zhang*
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*2*
Contribution to Project:	*Lihua carried out qPCRs validation of biomarkers.*
Funding Support:	

Name:	*Caroline Coletti*
Project Role:	*administrator*

Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	*1*
Contribution to Project:	*Caroline was responsible for IACUC and IRB protocol revisions, submissions, and compliance. She also coordinated patient sample collection.*
Funding Support:	

Name:	*Patricia Donahoe*
Project Role:	*mentor*
Researcher Identifier (e.g. ORCID ID):	0000-0001-5502-4497
Nearest person month worked:	*1*
Contribution to Project:	*Career mentoring.*
Funding Support:	

Name:	*Michael Birrer*
Project Role:	*Other mentor*
Researcher Identifier (e.g. ORCID ID):	0000-0002-3861-4521
Nearest person month worked:	*0*
Contribution to Project:	*Career mentoring.*
Funding Support:	

Name:	*Guangping Gao*
Project Role:	*Consultant, key personnel*
Researcher Identifier (e.g. ORCID ID):	0000-0003-0097-9012
Nearest person month worked:	*0*
Contribution to Project:	*Consulting on AAV vector design and production.*
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Added the following

OPP1203044 Pepin (PI)
Bill and Melinda Gates Foundation Grand Challenges Exploration Phase II 11/21/2018-10/31/2020
A Pipeline to Screen and Validate MISR2 Agonists as Contraceptives that Inhibit Primordial Follicles
The goal is to screen and identify more broadly applicable, improved, and less expensive small molecule contraceptive agents using a high-throughput cell-based screen.
Role: PI

- **What other organizations were involved as partners?**

Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**

Nothing to report.

9. **APPENDICES:**

Nothing to report.