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TITLE: AAV9 Gene Therapy using a Novel Engineered MIS to Treat Ovarian Cancer

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14. ABSTRACT

Ovarian cancer is the most lethal malignancy of the female reproductive tract with few treatment options. MIS has previously been shown to inhibit growth of a stem-like subset of the total cancer cell population in ovarian cancer lines. We have recently engineered novel peptide modifications to human MIS (LR-MIS), which increase production and potency in vitro and in vivo, and inserted it into an AAV9 vector. AAV delivered gene therapy has undergone a clinical resurgence with a good safety profile and sustained gene expression. Therefore, we propose to evaluate the efficacy of a single dose of AAV9-LRMIS to inhibit chemoresistant recurrences in a patient-derived ovarian cancer xenografts (PDX) model of surgical debulking followed by chemotherapy. To evaluate the potential responsiveness of the patient population and elucidate the mechanism of action we will analyze gene expression changes in primary cell lines treated with LRMIS.

15. SUBJECT TERMS

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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 apoptosis 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, significant progress was made on all 3 tasks. New patient samples were banked and assessed for tumor-forming abilities. Responsiveness to MIS was evaluated in 12 primary patient-derived lines using tumor spheroid (organoid) cultures treated with a dose response of recombinant MIS for 72h. Single-cell RNA-sequencing was performed on 3 patient samples and a primary cell line treated with MIS for 24h at 10ug/ml. Putative pathways and target genes downstream of MIS were identified and their expression, along with markers of stemness and epithelial-mesenchymal cell state were assessed in 12 primary patient derived lines using tumor spheroid cultures treated with recombinant MIS at 10ug/ml. A new model of surgical debulking was successfully evaluated in two different strains of immunocompromised mice with a luciferized patient-derived xenograft line to allow real-time monitoring in-vivo imaging of disease progression.

2) Specific objectives:

Task1:

1a- Submit any protocols or amendments to IRB committee. (month1-6)

Our IRB protocol is submitted and up to date. 100% completion.

1b- Collect and bank new patient samples, and derive new cell lines. (months 1-60)

The rate of new incoming patient samples has been low in the past year. We banked 4 new patient ascites, from which we derived at least 1 stable cell line, and tested 2 in vivo for tumorigenicity.

1c- Produce recombinant MIS necessary for planned experiments. (months 1-60)

Over 20mgs of recombinant MIS was produced over this period which was not limiting to any experiments we wanted to perform.

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)

We have performed dose-response experiments for MIS in 12 primary cell lines to date. We have similarly performed dose-responses to carboplatinum in 6 primary cell lines. We have not yet evaluated combination therapies (such as MIS+carboplatinum, or MIS+doxorubicin). 50% completion.

1e- Quantify rate of apoptosis in spheroid culture treated in vitro with MIS. (months 1-24)

The observed rate of apoptosis in preliminary experiments has been negligible (around 1%), we have however been evaluating viability using cell titer glo™ reagents which allows very precise quantification of the viability of spheroid cultures.

1f- Quantify the inhibition of stemness using in vitro assays. (months 1-24)

Inhibition of stemness is currently being evaluated using qPCR with validated markers of stemness. This analysis is currently under way with new and newly identified markers in 12 primary cell lines.

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

We have performed preliminary analysis of a panel of candidate biomarkers identified in 3 patient samples evaluated by single-cell RNA-sequencing. Particularly H19, SLPI, PI3 were validated in 12 primary cell lines.

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 optimizing siRNA treatment protocols to MISR2 which will be necessary to show specificity to the target gene and pathway. In particular we are comparing strategies involving siRNA pools transfected with cationic lipids versus new transfection-free siRNA technology (Dharmacon) which promises superior efficiencies in primary cell lines. If successful siRNA may represent a superior technique to shRNA for this objective.

Milestones:

1) To have determined the response rate, and dose sensitivity to MIS of at least 20-30 primary cell lines.

We have performed this analysis in 12 cell lines to date, and are on pace to complete this milestone within the first 24 months of the grant.

2) To differentiate cytostatic from cytotoxic effect of MIS, either alone or in combination with chemotherapy, particularly on the stem cell compartment.

We have evaluated the cytostatic and cytotoxic effect of MIS alone (user Cell Titer Glo) but we have not evaluated drug combinations using this assay. We have however begun to evaluate expression of stem cell markers following treatment with MIS, as well as performed single-cell RNA sequencing in 3 patient samples treated with MIS which allows putative stem cell populations to be specifically evaluated for response.

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 or rescue experiments in vitro.

Task2:

2a- Screen for expression of canonical MIS/MISR2 pathway genes. (months 1-24).

We have performed qPCR screens for all active variants of the receptor (MISR2v1, MISR2v2) in over 12 primary cell lines. We have also validated canonical downstream pathway genes (ID2, ID3, SMAD6) with and without MIS treatment (24h, 10ug/ml).

2b- Perform RNA-seq on at least 10 new primary cell lines. (months 1-24)

We have performed single cell RNA-seq (inDRO) in 3 patient samples treated with MIS (AV, AQ, BN), and one corresponding cell line (AQ-sph).

2c- Analyse RNA-seq data to identify novel responsive pathways and biomarkers. (months 24-36)

We have begun to analyze response pathways and biomarkers based on the analysis of our first cohort of 3 patients and expect to expand our analysis and perform more sophisticated pathway analysis as data allows. We have preliminarily identified a potential pathway related to TGF- β protease activation modulated by MIS (PI3, SLPI) and a long non-coding RNA involved in epithelial-mesenchymal differentiation (H19).

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 inDRO sequencing in 3 patient samples, and are planning to expand this panel using 10X genomic platform, which is a commercial version of droplet sequencing, for which we have recently acquired the machine (chromium controller), which should allow us to accomplish this goal.

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 or absence of all active forms of the receptor in our primary cell line panel (N=12) along with 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 single cell RNA seq in 3 samples to date, but have acquired and validated the technology to perform this analysis in house, thanks to the purchase of a 10X chromium controller.

3) Find new pathways regulated by MIS.

We have identified potential new pathways modulated by MIS including a putative TGF-beta induced protease pathway involving SLPI and PI3.

4) Perform Drop-seq on 3 patient lines that are highly responsive to MIS.

We have performed inDROP sequencing on 3 patient lines treated with MIS or vehicle control (2500 cells per sample) with high levels of expression of the MISR2 receptor. We will expand our analysis to the 10-30 proposed primary lines using another droplet-based sequencing technology (10X genomics).

Task3:

3a- Submit any protocols or amendments to IACUC committee. (months 1-6)

Our IACUC protocol is submitted and up to date. 100% completion.

3b- Evaluate model of surgical debulking + chemotherapy using previously characterized PDX model. (months 1-12)

We have evaluated and optimized a surgical debulking model using our AM luciferized PDX line, using both carboplatinum and parp inhibitor therapies. We found that R2G2 mice were had much better survival than standard NSG mice (greater than 80% versus 20%) for debulking surgery. However R2G2 mice have lower take-up rates of PDX implantations. Balancing these two attributes will be essential to evaluating treatments in the appropriate chemoresistant recurrence mouse model.

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 not yet tested AAV9-MIS in our surgical debulking model of chemoresistant recurrences.

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)

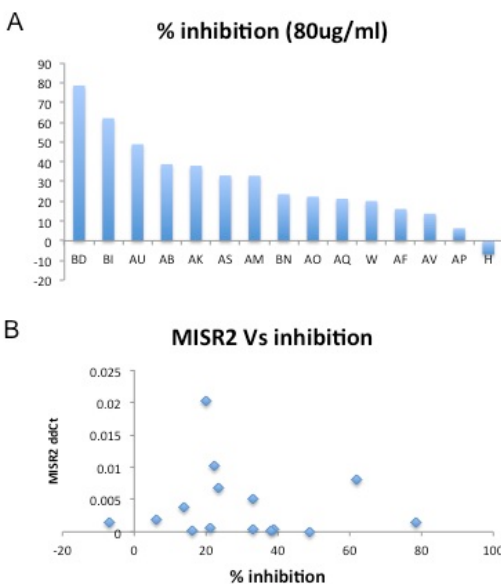


Fig1. Response to MIS does not correlate with receptor expression levels of 15 primary cancer cell lines.

During the reporting period of this award we collected ascites samples, and continued expanding our bank of patient samples that now exceeds 120 ascites. All samples were banked including the supernatant and the cellular component. For each of these samples we evaluate 4 different culture conditions to engineer stable cell lines for analysis in vitro and in vivo. We tested these cell lines for their tumorigenic capacity in mice, and their ability to form spheroids in vitro. We tested in this manner 15 patients for their responsiveness to MIS using an improved spheroid viability assay with a fluorimetric readout (Cell Titer Glo) with improved the reproducibility.

In this assay multiple spheroids (total of 5000 cells) are plated into wells of 96 well plates and treated with a range of recombinant MIS doses (0-80ug/ml). Thereafter, the total viability of the spheroids over 72h was accessed by fluorometry using a plate reader (luciferase). This allows for more precise measurement of the viable fraction of the spheroid cells. In our more dose-response fluorometric viability assay we assayed inhibition in 15 patient-derived cell lines. We observed variable inhibition starting at 20ug/ml (8.9%+/-19.7), and 80ug/ml (29.9%+/-19.6). Interestingly the most inhibited tertile, which we defined as “high responders” had on average 53% inhibition of viability by MIS at 80ug/ml compared to vehicle control, whereas the lower tertile “low responders” had only 9% inhibition at this dose. We used this cutoff to compare MISR2 expression and biomarker expression in subsequent analysis. We did not find any significant correlation between the level of expression of the MIS receptor (MISR2) and the response to its ligand (Fig1). Neither did we find any relationship to the canonical markers of response to MIS such as SMAD6, SMAD7, ID2, and ID3.

To investigate the effect of MIS on gene regulation, primary patient-derived spheroid cell lines (n=3 patients) were treated with rMIS (10ug/ml) or vehicle control and cultured 24h in ascites supernatant prior to being analyzed by inDROP sequencing (Fig2A). Single cell transcriptome analysis allowed us to investigate gene response in more detailed manner, which allowed us to uncover a number of potential pathways regulated by MIS (such as pathway of PI3-SLPI-WDFC2) which we are validating across a panel of patient-derived cell lines (N=15) (fig 2C). We also uncovered a high level of heterogeneity of cell states in each ascites samples with putative novel markers of distinct cell populations which may represent epithelial-mesenchymal cell states and stem cell markers. This technique also allowed us for the first time to observe both cancer cells and immune cells and their interaction in presence of MIS. Our preliminary analysis suggests a potential alteration of cell states in the T cells co-cultured with the cancer cells (Fig2B), when stimulated with MIS with important implications on MIS in immunology.

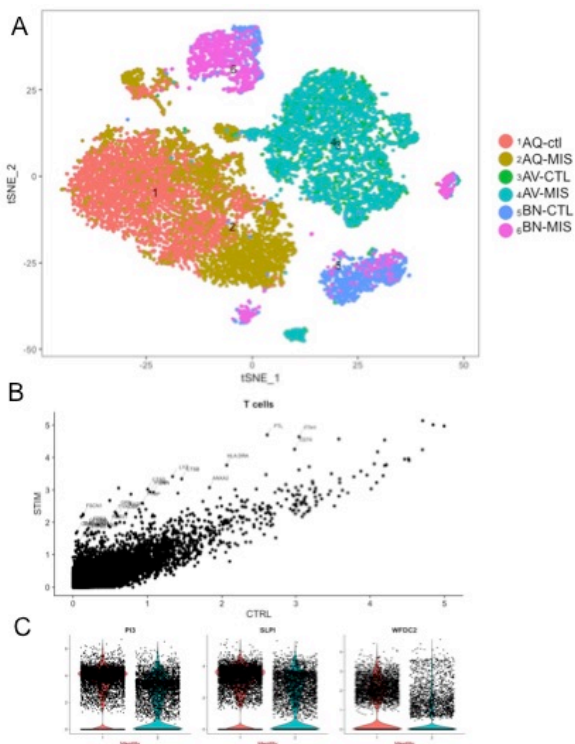


Fig2-DRIP-seq analysis. A) TSNE plot of 3 patient primary ascites treated with MIS or control. B) Alteration of expression of T cells by MIS in sample BN. C) violin plot of 3 candidate biomarkers regulated by MIS in sample AQ.

We believe that as we perform more scRNA-seq experiment on more patient cancer cells treated with MIS we will gain more statistical power and confidence in our ability to identify novel pathway candidates acting on the cancer stem cell which may allow us to not only predict a response rate but also predict the magnitude and the durability of the response.

Finally, we evaluated a novel chemoresistant recurrence model in which animals are surgically debulked and treated with chemotherapy and followed with in vivo imaging (luciferase) to monitor recurrences (**Fig3**). For this purpose we have been testing in vivo imaging using luciferized PDX models as well as optimized a surgical debulking protocol in anticipation of testing our gene therapy with MIS in our best responders. We have identified N2G2 as superior mouse strain for this model, with greater than 80% survivability to the surgery, however, tumorigenicity may be a limitation in this model that is less permissive than NSG mice.

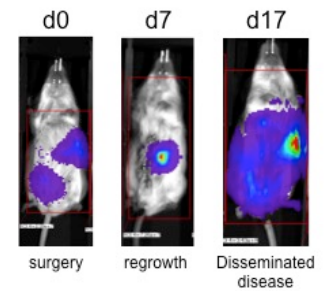


Fig3. A surgical debulking model of disseminated chemoresistant recurrent disease. Example of a luciferase producing ovarian cancer PDX model showing tumor recurrence following surgical debulking and Parp inhibitor treatment.

o **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 two 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 hope to present the work detailed in this proposal at Marsha Rivkin/AACR meetings in September following the Ovarian Cancer Academy meeting, for which I submitted an abstract. I am looking forward to the Academy meeting to interact with the other awardees, mentors, and the Deans and continue collaborations (such as with Anirban Mitra) or develop new collaborations.

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 met with my “Other Mentor” Michael Birrer to discuss the state of the art in ovarian cancer treatment. He has since relocated to the University of Alabama, where he is now director of the Cancer Center, but we have made arrangements to stay in contact with teleconferences. I have also established a new working relationship with the new director of Gynecologic Oncology, David Sprigs who has been very supportive of my work. Finally, 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 secured funding from a team grant to the OCRFA with David Sabatini and Peter Ghoroghchian 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 apply to independent “R” level funding by the next calendar year through the NIH, as well as submit a new manuscript on MIS signaling at the single cell resolution in ovarian cancer ascites.

Conferences:

12th Biennial Ovarian Cancer Research Symposium (Rivkin Center for Ovarian Cancer and the American Association for Cancer Research), September 13th-15th, 2018. TBD. 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 Nagykerly, Joy Poulo, Patricia K Donahoe, David Pepin.

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

I am hosting two high-school students (Selena Wu, Augustin Wright) in the laboratory this summer who are working on the ovarian cancer projects which is a great opportunity for them to be exposed to this type of work and increase their exposure to careers in the field of ovarian cancer research.

- **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 plan to increase the number of primary cell lines screened from 12 to 20 by the next reporting period. This will include not only responsiveness to MIS but also to first line chemotherapeutics (doxorubicin, carboplatin), and combination treatment in responders.

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 increase our expression signature screen by single cell RNA sequencing from 3 patients to 10 in the next reporting period. This will allow us to expand the statistical power and biological robustness of the dataset and allow us to identify additional biomarker candidates to validate.

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 can now proceed with evaluating gene therapy in our newly established surgical debulking recurrence model, which we have fully validated. We will proceed with the best responders identified in Aims 1 & 2.

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 single cell RNA-sequencing.

4. IMPACT:

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

Firstly, we have developed a new in vitro assay which allows the study of immune-cancer cell interactions at the single cell level by establishing a new short-term culture condition that closely mimics the peritoneal environment by using cleared ascites supernatant as a culture medium instead of chemically-defined media, and using disruptions in gene expression in distinct immune cell types and cancer cells as markers of response with single cell RNA-sequencing. This model is unique in allowing autologous interaction of immune and cancer cells from the same patient, in a culture medium that incorporates all the cytokines and growth factors present in the patient, and excludes artificial additives of commercial media which are known to alter metabolism (e.g. high glucose and pyruvate), which may affect interactions and behaviours of these cell types. We believe an assay such as this will likely enable us and others to better dissect heterogeneous patient response, and direct personalized treatment approaches.

Secondly, we have developed the first ovarian cancer patient-derived xenograft model that truly mimics patient care by incorporating adjuvant chemotherapy, surgical debulking, and disease burden monitoring with in vivo imaging to follow chemoresistant recurrences.

This model may be useful to the field of ovarian cancer research by incorporating important aspects of patient care that might influence treatment outcome and play a role in the biology of chemoresistance.

- **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:**

Nothing to report.

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. TBD. 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 Nagykerly, 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.**

Nothing to report.

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

Nothing to report.

- **Technologies or techniques**

Our newly developed assays (autologous ascites cultures with single cell sequencing, and surgical debulking chemoresistance PDX) will be presented at the upcoming 2018 Rivkin meeting (see conference presentations), and was presented to the other mentees of the Ovarian Cancer Academy as part of work in progress monthly teleconferences.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

- *biospecimen collections:*

We banked 4 new patient ascites, from which we derived at least 1 stable cell line, and tested 2 in vivo for tumorigenicity.

- *models:*

We have developed an ovarian cancer patient-derived xenograft model that mimics patient care by incorporating adjuvant chemotherapy, surgical debulking, and disease burden monitoring with in vivo imaging to follow chemoresistant recurrences.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

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

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

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

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

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

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

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

PI (David Pepin) has received a new grant in ovarian cancer research on a different unrelated project from the OCRFa

Ovarian Cancer Research Fund Alliance (OCRFA) 01/01/2018- 12/31/2021. Title: A Personalized Approach to Identify and Target Ovarian Cancer Liabilities. Our goal of this team grant is to identify new vulnerabilities in ovarian cancer cells by using CRISPR/Cas9 technology to systematically mutate every gene in a panel of ovarian cancer cells taken from patient tumors, which will allow us to identify new drug targets that can increase sensitivity to chemotherapy. Furthermore, we will work toward treating this disease using synthetic nanoparticles that target the vulnerabilities we identify, using a model system that closely mimics patient treatment in patient-derived xenograft models.

Role: co-PI

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

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES:

Nothing to report.