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TITLE: Pharmacogenomic signatures that predict drug response and resistance in high-grade serous ovarian cancer using patient-derived organoids and their exosomes

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
High-grade serous ovarian carcinoma (HGSC) is the most common and lethal subtype of ovarian cancer due to the advanced stage at diagnosis and high rate of relapse with platinum resistance. Currently, individualized therapy is unattainable due to limited knowledge of early markers of drug resistance. Repetitive tumor sampling is an invasive approach and fails to account for tumor heterogeneity and the evolution of drug resistance. Better models for identifying patients that may develop chemotherapy resistance are required to make progress in treating patients with ovarian cancer. *The hypothesis is that patient-derived organoids (PDOs) can be used to identify effective new therapies for HGSC and that analysis of responding and non-responding PDOs and their secreted exosomes can be used to identify markers that predict treatment response or resistance in patients.* This will be addressed with the following aims: Aim 1 will determine the sensitivity and resistance of HGSC PDO's to NCI-IND drugs as single agents and in combination with standard of care, Aim 2 will determine miRNA signatures that predict drug response from PDO's and their secreted exosomes, while Aim 3 will determine whether organoid response and drug response signatures from PDO's and their secreted exosomes correlate with actual patient response.

15. SUBJECT TERMS
Ovarian cancer, high-grade serous, precision medicine, drug resistance, biomarker discovery, patient-derived organoids, exosomes, liquid biopsy, high-throughput drug screen, 3D modeling

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1. INTRODUCTION:

Ovarian cancer is the fifth leading cause of cancer-related death among women and has the highest fatality rate among gynecologic cancers. In the United States, more than 20,000 new cases and 13,000 deaths from ovarian cancer occurred in 2017. High-grade serous ovarian carcinoma (HGSC) is the most common and the most lethal subtype of ovarian cancer. Although the 5-year survival rate has improved for patients diagnosed with advanced-stage HGSC, the long-term survival rate (>5 years) remains a meager 30%. This low overall survival is largely due to the advanced stage at diagnosis and a high rate of relapse due to the emergence of platinum resistance with few effective next-line treatments. At present, individualized therapy is limited due to poor access to repetitive tumor sampling and limited knowledge of early markers of evolving drug resistance. Tumor sampling is also an invasive approach and fails to account for the biologic adaptability of malignancies including tumor heterogeneity, stress response under the pressure of chemotherapy and the evolution of drug resistance. Better models for identifying patients that may develop chemotherapy resistance during treatment are required to make progress in treating patients with ovarian cancer. *The hypothesis is that patient-derived organoids (PDOs) can be used to identify effective new therapies for HGSC and that analysis of responding and non-responding PDOs and their secreted exosomes can be used to identify markers that predict treatment response or resistance in patients*. The hypothesis will be addressed with the following aims: **Aim 1** will determine the sensitivity and resistance of HGSC PDO's to NCI-IND drugs as single agents and in combination with standard of care, **Aim 2** will determine miRNA signatures that predict drug response from PDO's and their secreted exosomes while **Aim 3** determine whether organoid response and drug response signatures from PDO's and their secreted exosomes correlate with actual patient response.

2. KEYWORDS:

Ovarian cancer, high-grade serous, precision medicine, drug resistance, biomarker discovery, patient-derived organoids, exosomes, liquid biopsy, 3D modeling, high-throughput drug screen

3. ACCOMPLISHMENTS:

What were the major goals of the project?

	Timeline	Percentage of Completion
Specific Aim 1: Determine the sensitivity and resistance of HGSC PDO's to NCI-IND drugs as single agents and in combination with standard of care.		
Major Task 1: Conduct pilot drug screening study	Months	
Subtask 1. Local IRB approval and HRPO approval	1-3	IRB approved 03-26-2019, HRPO approved 08-06-2019
Subtask 2. Obtain tissue and blood samples from ovarian cancer patients; obtain clinical, pathological, and treatment history information.	3-14	30% complete
Subtask 3. Process patient tissues for PDOs. Perform drug screen on isolated PDOs with indicated drugs, identify lead compounds for each PDO, and isolate exosomes.	3-16	30% complete
Milestone(s) Achieved: Demonstrate sensitivity and resistance to drug/drug combination treatment in PDOs isolated from HGSC patients, and characterize PDOs and exosomes.	16	38% complete
Specific Aim 2: Determine miRNA signatures that predict drug response from PDO's and their secreted exosomes		
Major Task 2: Perform RNA-seq and whole exome sequencing analysis on PDOs and their secreted exosomes		
Subtask 1. Isolate RNA and DNA from parent tumor tissue, PDOs, and their secreted exosomes. Isolate exosomes and RNA and DNA from patient blood. Perform RNA-seq and whole exome sequencing on all samples.	16-18	0% complete

Subtask 2. Analyze whole exome sequencing data from parent tumors, PDOs, and patient blood samples, determine somatic mutations and perform hierarchical clustering analysis. Correlate drug response with tumor mutational status.	18-20	0% complete
Subtask 3. Analyze RNA-seq data for tumor subtyping, mRNA comparison between parent tumor and PDOs, identify predictive miRNA signatures from PDOs and exosomes for drugs tested, perform functional analysis of drug response signatures, validate signatures in independent patient sample set.	18-20	0% complete
Milestone(s) Achieved: Develop miRNA signatures that predict response to drug	20	0% complete
Specific Aim 3: Determine whether organoid response and drug response signatures from PDO's and their secreted exosomes correlate with actual patient response.		
Major Task 3: Correlate predictive drug response signatures to actual patient clinical response		
Subtask 1. Obtain treatment history and RECIST response from patients post-treatment with paclitaxel/carboplatin. Determine response to drugs included in the pilot screen if applicable.	20-22	0% complete
Subtask 2. Correlate patient clinical response with PDO response and miRNA signatures from PDOs and their exosomes.	20-24	0% complete
Milestone(s) Achieved:	24	0% complete

What was accomplished under these goals?

Major Task 1 under specific Aim 1 applies to this reporting period in accordance with the approved timeline in the Statement of Work.

Specific Aim 1: Determine the sensitivity and resistance of HGSC PDO's to NCI-IND drugs as single agents and in combination with standard of care.

Major Task 1: Conduct pilot drug screening study

Subtask 1. Local IRB approval and HRPO approval

OUHSC IRB approval was received on March 26, 2019 and HRPO approval was received on August 6th, 2019.

Subtask 2. Obtain tissue and blood samples from ovarian cancer patients; obtain clinical, pathological, and treatment history information.

The approved protocol was reviewed with the clinical and biospecimen teams at the Stephenson Cancer Center. Tissue and blood sample collection began in November 1st. The target enrollment for Year 1 Quarter 1 was 10 patients. A total of 0 patients were enrolled in Y1Q1 (Aug. 6th –Nov. 6th) given that the clinical team was not prepared to begin enrollment until November 1st. The target enrollment for Y1Q2 (Nov. 7th – Feb. 7th) was 11 patients and total of 2 patients were enrolled. The lack of enrollment was primarily due to the holidays as well as early preparations being made by the cancer center for the COVID-19 pandemic. The target enrollment for Y1Q3 (Feb. 8th – May 8th) was 10 patients and a total of 0 patients have currently been recruited. A "shelter-in-place" was ordered for Oklahoma City on March 16th and the OUHSC has placed a temporary hold on all tissue collection beginning March 17th. All non-essential research was put on hold and laboratories were shutdown beginning March 17th at OUHSC. Currently the OUHSC is scheduled to remain closed for all non-essential activity until May 8th so it is unlikely that any further patients will be recruited for this quarter.

Subtask 3. Process patient tissues for PDOs. Perform drug screen on isolated PDOs with indicated drugs, identify lead compounds for each PDO, and isolate exosomes.

Given the current circumstances and the collection of only a few tissue and blood samples, processing of the patient tissues has been placed on hold. Once tissues have been processed they need to immediately move on to drug screening and analysis which is approximately one month's work. With a significant amount of uncertainty regarding whether there would be enough time to complete the screen and analysis before laboratories were shut down, I have decided to preserve these patient tissues for a later time.

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

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

I will aggressively pursue patient tissue collection once suspension of tissue collection due to the COVID-19 pandemic is lifted. I will discuss ideas for increased enrollment with Stephenson Cancer Center clinical team once the "safer-at-home" order has been lifted in Oklahoma City. I may also consider the addition of a second site/collaboration with another university to increase tissue collection (with prior approval). All calibrations for the liquid handling robot and setup for the high-throughput drug screen will be done using cell lines in order to allow these processes to occur while the project is waiting on tissue collection.

4. IMPACT:

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

Nothing to Report

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:

Changes in approach and reasons for change

Nothing to Report

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.

This project relies on the collection of primary patient tissue which serves as the starting material for all further experiments. While HRPO approval was received on August 6th 2019, tissue collection wasn't up and running at the Stephenson Cancer Center until November 2019 leading to a three month delay. In addition, the COVID-19 pandemic has significantly impacted the ability to make progress on Major Task 1 which relies entirely on the collection of patient tissue for Subtasks 2 and 3. Tissue collection was disrupted late January / early February due to preparations for the pandemic at the Stephenson Cancer Center. Tissue collection was temporarily suspended officially on March 17th by the OUHSC IRB due to a "safer-at-home" order put in place by the governor of Oklahoma and the mayor of Oklahoma City. All non-essential research has been suspended and laboratories were ordered to shut down for an unknown period of time.

Once the "safer-at-home" order is lifted and laboratories are allowed to re-open, preliminary work to ensure that the liquid handling robot, high-throughput plate reader, and high-throughput imager are calibrated and in working order will proceed by utilizing cell lines for these procedures instead of patient tissue. I will meet with the clinical team to discuss ways to increase enrollment for tissue collection once the temporary hold on tissue collection has been listed by the OUHSC IRB.

Changes that had a significant impact on expenditures

Delays in accounting at OUHSC lead to a 2 and half month delay in funds being made available for this project which delayed expenditures as well as hiring personnel. While this project was HRPO approved on August 6th, funds were not made available until October 27th. In addition, the laboratory shutdown due to the COVID-19 pandemic has led to a temporary halt on expenditures for this project from approximately January – April 2020 (Quarters 1 and 2). Once laboratories are allowed to re-open, expenditures will continue, if not increase in order to make up for the time that has been lost.

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

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Not applicable – no vertebrate animals are used in this project.

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

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

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**

Nothing to Report

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

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Kelsi Andrade
Project Role:	PI
Researcher Identifier:	0000-0001-6755-2297
Nearest person month worked:	11
Contribution to Project:	Drafted and submitted documents for committee approvals, setup clinical team for patient tissue collection
Name:	Daniel Andrade
Project Role:	Co-Investigator
Researcher Identifier:	0000-0002-0242-4785
Nearest person month worked:	11
Contribution to Project:	Performed experiments for equipment calibration, designed setup for high-throughput drug testing

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART:

Pharmacogenomic signatures that predict drug response and resistance in high-grade serous ovarian cancer using patient-derived organoids and their exosomes

OC180279

PI: Kelsi Andrade **Org:** University of Oklahoma Health Sciences Center **Award Amount:** $365,500

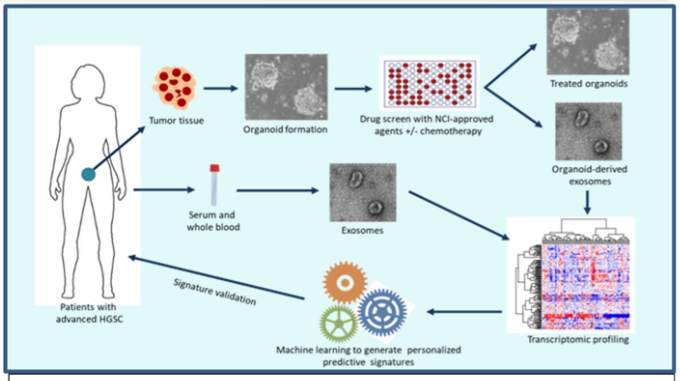
Hypothesis and Specific Aims

Hypothesis: Patient-derived organoids (PDOs) can be used to identify effective new therapies for HGSC and analysis of responding and non-responding PDOs and their secreted exosomes can be used to identify markers that predict treatment response or resistance.

- **Aim 1:** Determine the sensitivity and resistance of HGSC PDO's to NCI-IND drugs as single agents and in combination with standard of care
- **Aim 2:** Determine miRNA signatures that predict drug response from PDO's and their secreted exosomes
- **Aim 3:** Determine whether organoid response and drug response signatures from PDO's and their secreted exosomes correlate with actual patient response

Approach

PDOs from 21 untreated, treated, and resistant HGSC patients will be used to conduct a pilot study of NCI-IND drugs as single agents and in combination with standard-of-care. We will perform RNA-seq analysis to determine individual drug response signatures from both organoids and their secreted exosomes. We will correlate organoid response and drug response signatures from both the organoids and their secreted exosomes to actual patient response. Treatment of untreated PDOs with standard-of-care therapy will allow for clinical validation of signatures.



Accomplishment: IRB approval was received 03-26-2019; HRPO approval was received 08-06-2020. Patient tissue collection began 11-01-2019.

Timeline and Cost

Activities CY	19	20
Conduct pilot drug screening study		
RNA-seq and whole exome sequencing analysis on PDOs and their secreted exosomes; correlate with actual patient response		
Estimated Budget ($K)	**$163,994**	**$198,506**

Updated: 4-14-2020

Goals/Milestones

CY19 Goal – Demonstrate sensitivity and resistance to drug treatment in PDOs isolated from HGSC patients and characterize PDOs and their exosomes.

- ❑ Conduct pilot drug screen with PDOs and isolate exosomes

CY20 Goals – Develop miRNA signatures that predict response to drugs and correlate with actual patient response.

- ❑ Investigate earplug designs based on collected features
- ❑ Complete formal attenuation and comfort trials of earplugs

Comments/Challenges/Issues/Concerns

- The COVID-19 pandemic has significantly impacted the ability to collect patient tissue and conduct research due to a temporary halt by the OUHSC IRB and laboratory shutdowns at OUHSC.
- Expenditures are significantly reduced temporarily due to laboratory shutdown

Budget Expenditure to Date

Projected Expenditure: $163,994.00

Actual Expenditure: $36,881