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TITLE: Developing Clinically Relevant Models of Mucinous Ovarian Carcinoma for Testing Therapies

PRINCIPAL INVESTIGATOR: Dr. Kylie Gorringe

CONTRACTING ORGANIZATION: The University of Melbourne (Peter MacCallum Cancer Centre)

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**6. AUTHOR(S)**
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**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

**The University of Melbourne**
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**14. ABSTRACT**
This project aims to create patient-relevant models for mucinous ovarian cancer (MOC). Over the last year, we have begun PDX experiments, transplanting one MOC case subcutaneously (no growth after 6 months), intraperitoneally (growth observed within 2 months) and intra-bursally (growth observed within 2 months). We continue to collect tissue, although the pace has slowed, likely due to COVID disruptions at two collection sites. Nonetheless we have received four new carcinomas and one borderline tumour in the last year. Two of the carcinomas have grown sustainably as organoid lines. No cell lines have yet been able to be derived.

**15. SUBJECT TERMS**
Ovarian Cancer, patient models, mouse xenografts, DNA sequencing

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
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## 1. Introduction

Mucinous ovarian cancer is a rare ovarian cancer subtype with a very poor prognosis; median survival for stage III/IV disease is less than 15 months. Advances in mucinous ovarian cancer treatment options have been hindered by a lack of appropriate models for use in research laboratories. This grant aims to develop patient-derived laboratory models of mucinous ovarian cancer that accurately represent the human disease. Using these clinically relevant models we can test novel drugs and combination therapies in a controlled setting, providing us with more confidence that these treatment modalities will be successfully translated to the clinic.

## 2. Keywords

Mucinous, ovarian, cancer, rare, patient-derived, models, xenograft, drug, therapies, treatment, organoid

## 3. Accomplishments

*What were the major goals of the project?*

| **Major Task 1** | Months | % completed |
|---|---|---|
| Subtask 1: Obtain HRPO approval for human tissue use | 1-6 | 100 |
| **Specific Aim 1: To determine the optimal conditions under which MOC and borderline tumors can be cultured as organoids** | | |
| **Major Task 2** | Months | |
| Subtask 1: Obtain fresh tissue from first 10 patients and test multiple conditions | 1-12 | 100 |
| Subtask 2: Obtain fresh tissue from second 10 patients and test refined set of conditions based on the outcome of Subtask 1. | 10-20 | 70 |
| Subtask 3: Characterize successful organoids for morphology and growth rates | 13-24 | 50 |
| ***Milestone#1 Establish the optimal conditions for growth of MOC as organoids*** | 20-24 | 75 |
| **Specific Aim 2: To determine the optimal conditions under which MOC can be cultured as stable long-term cell lines** | | |
| **Major Task 3** | | |
| Subtask 1: Obtain fresh tissue from first 5 patients and test multiple conditions | 1-12 | 100 |
| Subtask 2: Obtain fresh tissue from second 5 patients and test refined set of conditions based on the outcome of Subtask 1. | 10-20 | 0 |
| Subtask 3: Characterize successful cell lines for cell morphology and growth rates | 13-24 | 0 |
| ***Milestone#2 Establish the optimal conditions for growth of MOC as cell lines*** | 20-24 | 0 |
| **Specific Aim 3: To determine the optimal conditions under which MOC can be cultured as patient-derived xenografts** | | |
| **Major Task 4** | | |
| Subtask 1: Submit documents for Animal Ethics review.<br>• Submission of institution approved animal protocols and related material for DoD's ACURO approval.<br>Receive ACURO approval before initiating animal experiments. | 1-6 | 100 |
| Subtask 2: Obtain fresh tissue from first 5 patients and test multiple conditions<br>[18 mice per case x 5 = 90 mice] | 6-18 | 30 |
| Subtask 3: Obtain fresh tissue from second 5 patients and test refined set of | 13-24 | 0 |

| | | |
|---|---|---|
| conditions based on the outcome of Subtask 1.<br>[10 mice per case x 5 = 50 mice] | | |
| Subtask 4: Characterize successful PDX for morphology and growth rates | 20-24 | 0 |
| Subtask 5: Harvest tumors from successful PDX, store some tissue and passage remainder into new mice<br>[6 mice per case x 10 = 60 mice (at most)] | 12-24 | 0 |
| ***Milestone#3 Establish the optimal conditions for growth of MOC as PDX*** | 20-24 | 10 |
| **Specific Aim 4: To undertake genomic and immunohistochemical profiling of successful models and compare to the primary tumor.** | | |
| Major Task 5: Characterization of primary tumors | | |
| Subtask 1: Obtain frozen tissue from primary tumors and extract DNA after microdissection | 1-20 | 50 |
| Subtask 2: Obtain formalin-fixed paraffin embedded tissue from primary tumors and perform immunohistochemistry for tumor markers CK7, CK20, ER, P53, VSIG1 and HER2. | 1-20 | 50 |
| Major Task 6: Characterization of successful models | | |
| Subtask 1: Extract DNA from successful cell lines, organoids and PDX tissue | 18-20 | 30 |
| Subtask 2: Perform short tandem repeat profiling of tumors and models to validate identity | 20-22 | 30 |
| Subtask 3: Prepare formalin-fixed paraffin embedded tissue or cells from successful cell lines, organoids and PDX tissue and perform immunohistochemistry for tumor markers CK7, CK20, ER, P53, VSIG1 and HER2. | 20-24 | 30 |
| Subtask 4: Perform OPAL staining for immunological markers. | 20-24 | 0 |
| Subtask 5: Send DNA from primary tumors and successful models for whole genome sequencing | 20-22 | 30 |
| Subtask 6: Analysis of whole genome sequencing data | 22-24 | 30 |
| ***Milestone#4 Author manuscript(s) on the optimal conditions for development of MOC models and describe the successful models in detail.*** | | 0 |

## *What was accomplished under these goals?*

1. Major Task 1. Obtain HRPO approval for human tissue use

Relevant Human Ethics documents were first submitted online in June 2018. Approval was obtained in January, 2020

2. Major task 2. Organoids have successfully been grown from five carcinomas and five borderline mucinous tumours, establishing two different media conditions under which cells grow. Some samples grow best in one medium, and others in a different medium. We have also optimised conditions with which to freeze and recover the organoid lines.

3. Major task 3. Three samples have been attempted to grow as cell lines, but no success has been achieved as yet.

4. Major task 4. One carcinoma has been transplanted into 9 NSG mice (3 different cell numbers) subcutaneously (SC). The first 6 have not shown any sign of growth after 27 weeks, the last 3 have not shown growth after 8 weeks (ongoing). As an alternative route, organoid lines have been transformed with a luciferase vector to enable in vivo imaging to track growth. Four mice each have been transplanted intraperitoneally (IP) and intrabursally (IB), with 3 and 2 mice respectively showing growth at 4 weeks (ongoing). Another MOC case has been transplanted SC (3 mice) and IB (5 mice) but it is too early to know if this has been successful (5 weeks). Two additional cases have been transformed with the luciferase vector and are being prepared for transplant.

5. Major tasks 5 and 6.

Progress on these tasks indicated below. DNA was successfully extracted from all but 1 recent organoid line. Only three of the tissues have had enough DNA to send for sequencing, and in 1 case there was no tumour in the frozen and insufficient in the FFPE piece (*). IHC is completed for all with available tissue/organoids for CK7, ER, p53, Ki67 and ERBB2. OPAL and STR sequencing will be done when the cohort is complete.

| Type | DNA T | DNA O | DNA seq T/O | IHC T | IHC O |
|---|---|---|---|---|---|
| Borderline | N | Y | N | Y | Y |
| Borderline | N | Y | N | Y | Y |
| Borderline | N | Y | N | Y | Y |
| Borderline* | N | Y | Y (O only) | N | Y |
| Borderline | N | Y | N | Y | Y |
| MOC | Y | Y | Y | Y | Y |
| MOC | Y | Y | Y | Y | Y |
| MOC | Y | Y | Y | Y | Y |
| MOC | Y | Y | N | Y | Y |
| MOC | N | N | N | Y | N |

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

A graduate research student (Masters of Biomedical Science) and a PhD student have been working on this project as part of their training. The Masters student completed their degree and has now started a PhD, and the PhD student has submitted their thesis. The postdoc recruited to the project in the last year has undertaken training to learn new PDX techniques.

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

A presentation at the Ovarian Cancer Australia symposium by the PI disseminated the results of the study to clinicians, researchers and consumers who attended. The PI also presented an update at the Victorian Comprehensive Cancer Centre Gynae Stream Research Meeting.

Both PhD and Master's students presented their work at the Australia & New Zealand Gynae-Oncology Group Annual Scientific Meeting 2021.

The postdoc presented her work at the Canadian Conference of Ovarian Cancer Research

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

In the next reporting period we expect to finish establishing organoid cultures for all 20 patients (Major Task 2 complete) and will continue to try and establish 2D cell lines (Major Task 3, subtask 1). We expect to have tested the first 5 patient PDX models (Major Task 4, subtask 2). All immunohistochemistry should be completed on the primary tumours (Major Task 5 complete) and organoids (Major Task 6), and whole genome sequencing also completed for tumours, organoids and some PDX.

## 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 new to report

### *Actual or anticipated problems or delays and actions or plans to resolve them*

Continued COVID-19 outbreaks have slowed tissue collection for new cases and delayed the onset of PDX experiments since the animal house requested that no new mouse experiments be initiated during periods of high risk. This has been ameliorated recently by institutional changes to procedures whereby fully trained and vaccinated staff are able to undertake essential small-scale mouse experiments for existing grants (which we are thus able to do). We are reliant on our clinical colleagues for tissue collection, and unfortunately have no control over Department of Health restrictions regarding staff allowed on the site of surgery or reduction in surgeries. However, since we are very close to completing the 20 cases, we anticipate being able to finish the proposed work in the time remaining.

### *Changes that had a significant impact on expenditures*

The delay in funds being approved for use at the host institution and the delay in human ethics approval from HRPO.

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

Nothing to report

### *Significant changes in use or care of human subjects*

Nothing to report

### *Significant changes in use or care of vertebrate animals.*

Nothing to report – as mentioned in the 2020 report we amended the animal ethics to enable intra-bursal and luciferase monitoring, and these changes were approved by ACURO.

## *Significant changes in use of biohazards and/or select agents*

Nothing to report

## 6. PRODUCTS:

## *Publications, conference papers, and presentations*

Report only the major publication(s) resulting from the work under this award.

In the last 12 months the work was selected for an oral presentation at the Australian and New Zealand Gynaecologic Oncology Group's Annual Scientific Meeting (Science Symposium) and the South Australian Organoids Symposium (both national). Aspects of the work were also presented as a poster at the Canadian Conference for Ovarian Cancer Research (international). The CI also presented the work to the Victorian Comprehensive Cancer Centre Molecular Tumour Board and VCCC Gynae Research Group (local) and the Ovarian Cancer Australia Symposium (national).

## *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*

A REDCap database has been set up for internal use by our laboratory. It will be used to store pathology information related to the samples received into the laboratory. The REDCap database will also be used to track what applications each tissue will be used for (grown as organoids, snap frozen, fixed in formalin etc).

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

| | |
|---|---|
| Name: | Associate Professor Kylie Gorringe |
| Project Role: | PI |
| Researcher Identifier (e.g. ORCID ID): | ORCID ID: 0000-0001-5681-2022 |
| Nearest person month worked: | 3 |
| Contribution to Project: | A/Prof Gorringe wrote the human ethics application which was approved by the HRPO in January 2020. A/Prof Gorringe has supervised the project overall |
| Funding Support: | New Funding support:<br>NHMRC Ideas Grant (2021-22) no overlap<br>Cancer Australia/NBCF grant (2021-2024) no overlap |

| | |
|---|---|
| | DoD Teal Expansion award (2021-2023) is a further development of the current project in which the models developed in this award will be used to test therapies. |

| | |
|---|---|
| Name: | Dr Suad Abdirahman |
| Project Role: | Postdoctoral Scientist |
| Researcher Identifier (e.g. ORCID ID): | NA |
| Nearest person month worked: | 10 |
| Contribution to Project: | Dr Abdirahman has taken over processing tissues and culturing the organoids. She has carried out laboratory experiments to introduce the luciferase construct into the organoids, and has performed xenograft experiments. |
| Funding Support: | No change |

| | |
|---|---|
| Name: | Ms Olivia Craig |
| Project Role: | Graduate Student |
| Researcher Identifier (e.g. ORCID ID): | NA |
| Nearest person month worked: | 4 |
| Contribution to Project: | Ms Craig has assisted with sample processing and culturing of organoids |
| Funding Support: | No change |

| | |
|---|---|
| Name: | Professor Clare Scott |
| Project Role: | Co-investigator |
| Researcher Identifier (e.g. ORCID ID): | ORCID ID: 0000-0002-3689-5956 |
| Nearest person month worked: | 1 |
| Contribution to Project: | Intellectual contribution and training for Dr Abdirahman in the generation of PDX models. |
| Funding Support: | New Funding support:<br>-NHMRC Investigator grant (2022-2026) no overlap |

| | |
|---|---|
| Name: | Professor Rob Ramsay |
| Project Role: | Co-Investigator |
| Researcher Identifier (e.g. ORCID ID): | ORCID ID: 0000-0001-5003-0433 |
| Nearest person month worked: | 1 |
| Contribution to Project: | Intellectual |
| Funding Support: | No change |

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

**New funding support for PI:**
NHMRC Ideas Grant (2021-22) no overlap
Cancer Australia/NBCF grant (2021-2024) no overlap
DoD Teal Expansion award (2021-2023) is a further development of the current project in which the models developed in this award will be used to test therapies.
**New funding support for CoI Clare Scott**
National Health and Medical Research Council Investigator grant (2022-26). No overlap with current project.

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

Nothing to Report

## 8. SPECIAL REPORTING REQUIREMENTS

### *COLLABORATIVE AWARDS:*

Nothing to report

### *QUAD CHARTS:*

Nothing to report

## 9. APPENDICES: