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14. ABSTRACT

This project aims to create patient-relevant models for mucinous ovarian cancer (MOC). Over the course of the project, we have successfully developed multiple MOC and borderline tumour organoid lines able to be maintained in culture over the long term from. One of three attempts at growing 2D cell lines directly from tumour tissue were successful. Four organoid lines have also been converted to 2D cell lines (including the same one that was successful from tissue). We have tested 6 cases as PDX, some by tissue implantation but others using organoid cell suspensions, and evaluating 3 sites. None have grown at the subcutaneous site, however, both intra-peritoneal and intra-bursal routes have had some success.

15. SUBJECT TERMS

Ovarian Cancer, patient models, mouse xenografts, DNA sequencing

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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	100
Subtask 3: Characterize successful organoids for morphology and growth rates	13-24	100
Milestone#1 Establish the optimal conditions for growth of MOC as organoids	20-24	100
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	100
Subtask 3: Characterize successful cell lines for cell morphology and growth rates	13-24	40
Milestone#2 Establish the optimal conditions for growth of MOC as cell lines	20-24	50
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. • Submission of institution approved animal protocols and related material for DoD's ACURO approval. Receive ACURO approval before initiating animal experiments.	1-6	100
Subtask 2: Obtain fresh tissue from first 5 patients and test multiple conditions [18 mice per case x 5 = 90 mice]	6-18	100
Subtask 3: Obtain fresh tissue from second 5 patients and test refined set of conditions based on the outcome of Subtask 1. [10 mice per case x 5 = 50 mice]	13-24	0
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 [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	50
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	90
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	70
Major Task 6: Characterization of successful models		
Subtask 1: Extract DNA from successful cell lines, organoids and PDX tissue	18-20	70
Subtask 2: Perform short tandem repeat profiling of tumors and models to validate identity	20-22	0
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	70
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	90
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.		20

What was accomplished under these goals?

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

Relevant Human Ethics approvals were obtained.

2. Major task 2. Organoids

Specific Aim 1: To determine the optimal conditions under which MOC and borderline tumors can be cultured as organoids

Methods and Results:

a) Sample processing and passaging: The method is summarised in Figure 1. There was a steep learning curve in this processing procedure, however we had assistance from Co-I Ramsay's lab. Initially, we received any sample the was suspected of being mucinous histology. However, we found that we received tissues where the final histopathology was benign or not mucinous (eg endometrioid, sero-mucinous). Therefore, we refined our inclusion criteria to only those patients with at least mucinous borderline histology on intra-surgical frozen section, or a recurrent tumour with documented primary mucinous borderline or carcinoma. This measure reduced the benign samples to none, and reduced the incorrect histologies. We sometimes still occasionally get either endometrioid ovarian or metastases from colorectal tumours, however, generally the "hit" rate is better (Figure 1).

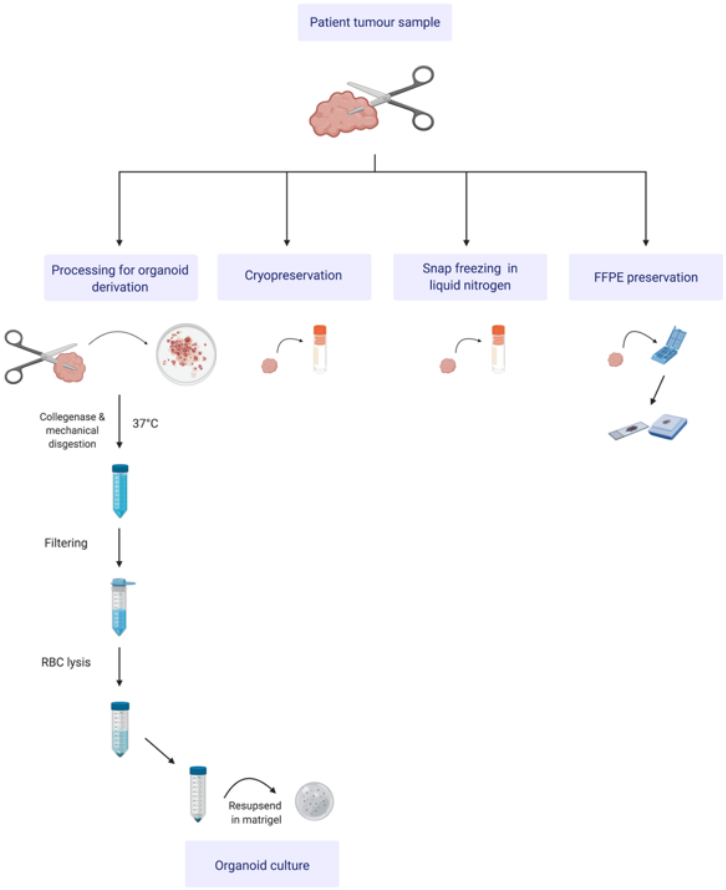
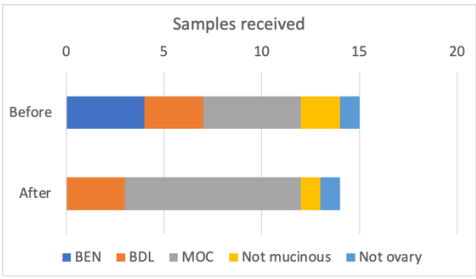


Figure 1: Overview of processing new tissues – samples are collected in media, processed to single cells for organoids and if enough also for 2D cell lines; remaining tissue is cryopreserved for future attempts and/or PDX, snap frozen for DNA/RNA extraction and formalin-fixed and paraffin embedded for histology. We prioritise organoids and cryopreservation, as DNA/RNA/histology can be performed on diagnostic tissue if necessary. Graph below shows improvement in desired histology before and after changing our collection procedures. BEN – benign, BDL – borderline, MOC – mucinous ovarian carcinoma.



b) Culture conditions and passaging: The most challenging aspect was the optimisation of media to grow organoids in. We tested media from colorectal and pancreatic organoids, and then modified the pancreatic media with different growth factors determined from our understanding of MOC biology (Figure 2). A paper

was recently published of ovarian cancer organoid culture, with a medium slightly different to ours (Kopper et al., Nature Medicine, 2019;25(5):838-49). We found that some of our organoids grew better in this medium, others worse and for others it made no difference (Figure 3). Therefore, we now grow new cases in both of these media and see which works best for that particular tumours.

Figure 2. Culture optimisation. A. Comparison of mechanical and enzymatic passaging. Mechanical passaging was better for organoid number, however at this point culture medium was sub-optimal. B. Comparison of Colorectal media and a pancreatic-style media, showing slight improvement with the pancreatic conditions. C. Testing various culture additions, showing that the addition of FGF2 and Noggin improved the number of organoids obtained. D. Comparison of 5 different media formulations, showing that ORGM3, which contained Noggin, FGF2, Wnt and R-spondin, was optimal for organoid growth. E. We subsequently tested whether the concentration of Wnt-3 could be reduced due to the high cost of this reagent, however it was clear that this was an essential ingredient for these tumours. Organoid images afer 3 and 4 passages in media with the usual vs half the usual Wnt3 concentration.

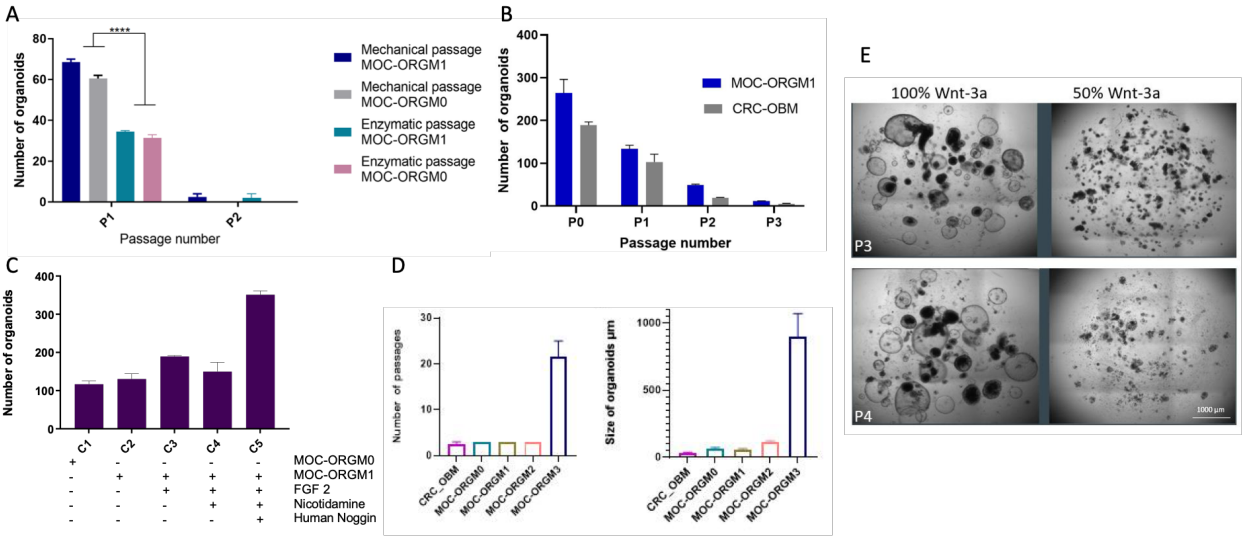
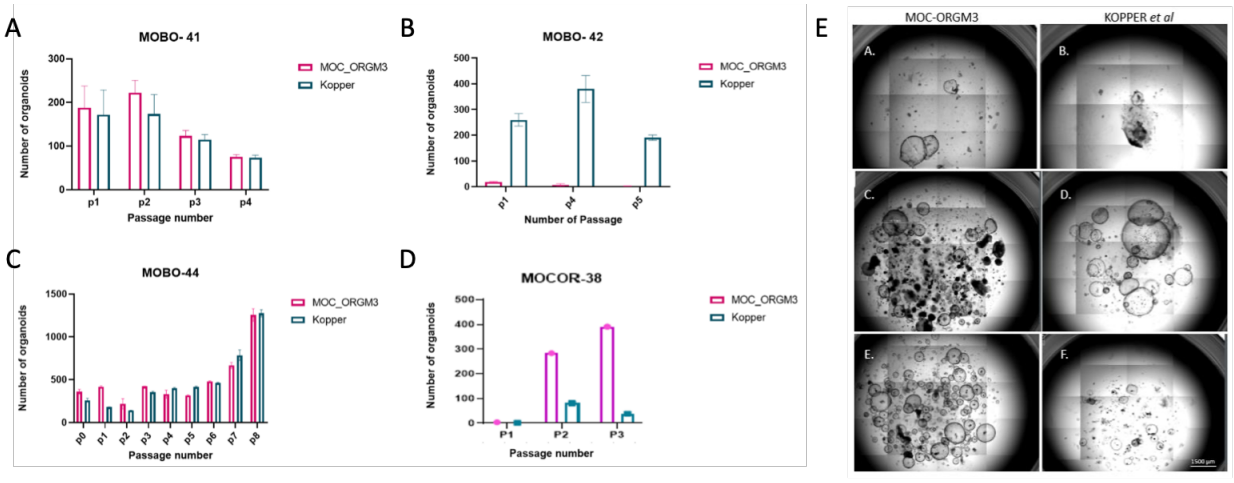
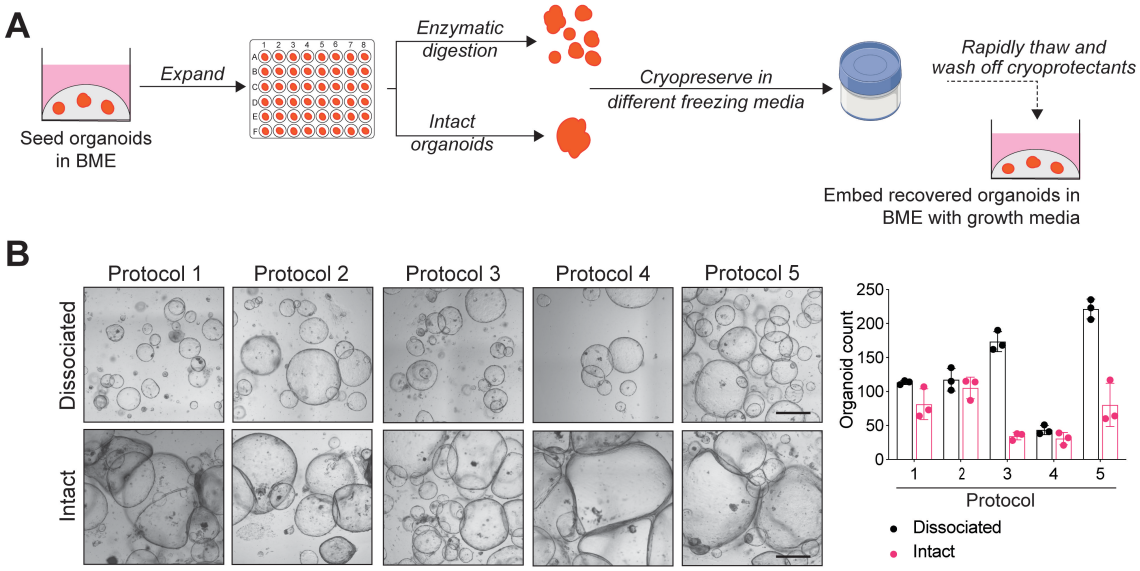


Figure 3. Comparison of our media vs media from Kopper et al. A, C. Cultures #41 and #44 did not show much difference between media, however #42 (B) grew better in Kopper media, while #38 (D, E) grew better in our media.



c) Culture freezing: We conducted an experiment to compare freezing media (Figure 4) and determined that freezing dissociated organoids in either 90% growth media/10% DMSO or a commercial Cell Recovery product (Thermo Fisher) gave the best recovery rates. We now freeze early passages in the commercial media and later, less precious passages, in the cheaper media/DMSO mix.

Figure 4. Successful re-establishment of MOC organoids utilising different cryopreservation media and processing conditions. (A) An established and well characterised MOC organoid line (#38) was selected to compare different cryopreservation conditions (1) cryopreserving fully intact organoids or after enzymatic digestion; or (2) in freezing media from any of the 5 protocols at passage endpoint. (B) Representative brightfield images of MOC organoids cryopreserved after revival in different freezing media (protocols 1-5) with quantification indicated on the right. Scale bar: 2000 µm. Data represent mean ± SD from triplicate wells. 1. 45% FCS, 45% growth media, 10% DMSO; 2. 90% FCS, 10% DMSO; 3. 90% growth media, 10% DMSO; 4. CryoStor CS10; 5. Recovery cell culture freezing medium



Conclusion: Organoids have successfully been grown from eight 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. We continue to collect tissue samples for processing and organoid establishment, with a current success rate of ~80% for more recent tissue collections.

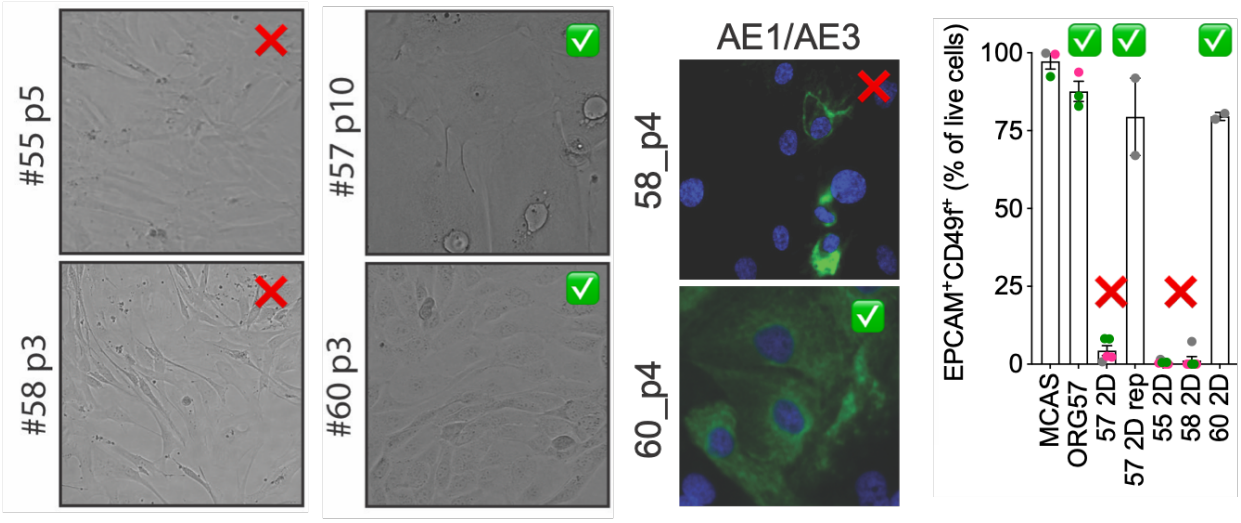
3. Major task 3. Cell lines

Specific Aim 2: To determine the optimal conditions under which MOC can be cultured as stable long-term cell lines

Methods: after tissue processing as for organoids above, cells were plated into 48 well plates using RPMI or DMEM media with 10% FCS as standard for 2D cell lines. Later we tested a culture media established by others for ovarian cancer cell culture, OCMI (Ince *et al.*, Nature Communications 6: 7419 (2015)). Additionally, we attempted to convert successful organoid cultures to 2D cultures by plating single-cell suspensions in OCMI. Cell morphology was assessed by bright-field imaging, immunofluorescence (pan-cytokeratin markers AE1/AE3 and flow cytometry with EpCam and CD49, Figure 5).

Results: Cell lines were attempted from 3 borderline tumours and 7 MOC. No attempts with RPMI or DMEM media were successful in the long term. The most common outcome for primary tissue cultured in these media was overgrowth of epithelial cells by fibroblasts (4/5 eg #55, 58, Figure 5), or death of the cells (1/5). One of three primary tissues cultured in OCMI is growing epithelial cells to passage 5 (#60), the other two look to be fibroblasts although this has yet to be confirmed (at p0 (#62) and p1 (#63)). Two of four attempts from organoid cultures in OCMI have reached passage 2-3 (#57, 60) and are confirmed to be epithelial cells.

Figure 5. Characterisation of 2D cultures. Left: brightfield images, Mid: pan-cytokeratin immunofluorescence, epithelial cells will have consistent cytoplasmic staining. Right: FACS of cultures with EpCam and CD49, % of live cells that are double positive (ie. epithelial) is shown. Green ticks show cultures with expected epithelial characteristics, red crosses show fibroblast characteristics.



Seven other tumours were also attempted however two of these turned out to be endometrioid not mucinous histology, and 5 had a final benign diagnosis. Two of the benign tumours did grow for 3-4 passages but were frozen down once the final diagnosis was obtained.

Conclusion: We conclude that establishing 2D cultures directly from primary tissue is challenging due to the overgrowth by fibroblasts, however the OCMI media is better than other media formulations. Adapting organoid cultures to 2D using OCMI appears to be a better method and we will be using this henceforth. We will continue to test and refine our procedures as we clearly do not have optimal conditions established yet. An additional possibility, yet to be tested, will be to establish cell lines from any successful PDX models.

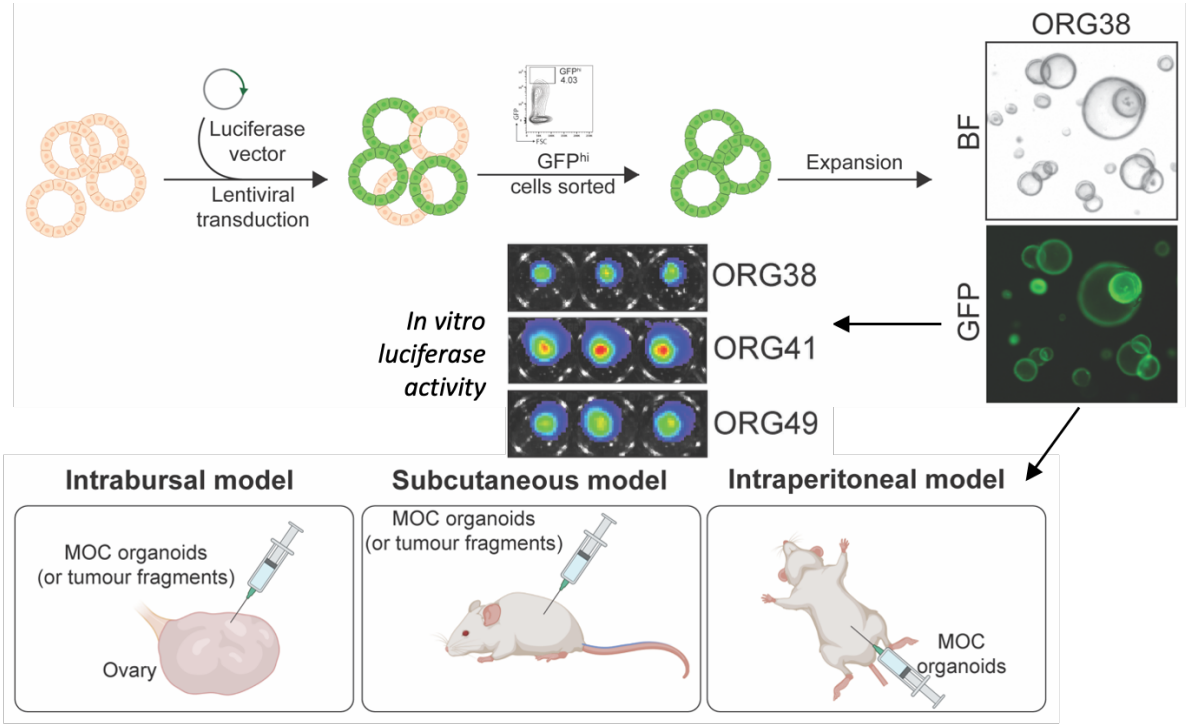
4. Major task 4. PDX

To determine the optimal conditions under which MOC can be cultured as patient-derived xenografts

Methods:
a) Tissue for transplantation: Two sources of tumour are used.
1) Because of the challenges in not having an accurate diagnosis when tissue is collected (this requires pathologist review), we are unable to use fresh tissue, therefore we attempted with cryopreserved primary tissue.
2) Organoid lines either original or transformed with a luciferase vector to enable in vivo imaging to track growth were tested (Figure 6). Four organoid lines have been transduced and three tested in vivo.

b) Routes of implantation: Three routes have been attempted – subcutaneous (SC), intra-peritoneal (IP) and intra-bursal (IB). Different numbers of cells implanted were tested.

Figure 6. Overview of PDX methods. Transduction of organoids with luciferase/GFP vector, which is then implanted into immune-compromised mice via one of 3 routes. Untransduced cryopreserved tissue can also be implanted, but is harder to track when using the IB or IP routes.



Luciferase reporter activity is assessed by *in vivo* imaging after intraperitoneal injection of D-Luciferin

Results:

1) Subcutaneous: Five carcinomas have been transplanted (1 testing 3 different cell numbers). None have grown after 3-12 months (Table 1)

Table 1	**Number of mice**	**Successful**	**Time-frame**
Sample	SC - organoid cells		
#38	n=9 (3 different cell numbers)	0/9	8-12 months
#41	n=5	0/5	3 months
#49	n=5	0/5	3 months
	SC - cryopreserved tissue		
#52	n=3	0/3	8 months
#55	n=5	0/5	6 months

2) Intraperitoneal: Three attempts have been made, all using organoid cells (Table 2, Figure 7). At this stage it is uncertain whether any have tumours growing. We have observed that the luciferase signal can be sustained for up to 3 months and then decrease thereafter, indicating perhaps cell stasis followed by eventual death.

Table 2

IP - luciferase-transduced organoids			
Sample	**Number of mice**	**Successful**	**Time-frame**
#38	n=4	0/4	6 months
#41	n=5	1/5*	3 months
#49	n=5	4/5*	3 months

*retain luciferase signal, however this doesn't mean the tumour is growing at this still relatively early timepoint.

3) *Intrabursal*. Five attempts have been made, three with luciferase-transduced organoids and two from cryopreserved tissue (Table 3, Figure 7). The earliest attempt looks to have one successful mouse, with a luciferase signal emerging at 5 months and growing until the current timepoint of 6 months. True success will be determined when the mouse is harvested.

Table 3

IB - luciferase-transduced organoids			
Sample	**Number of mice**	**Successful**	**Time-frame**
#38	n=9	1/9	6 months
#41	n=5	4/5*	3 months
#49	n=5	3/5*	3 months

*retain luciferase signal, however this doesn't mean the tumour is growing at this still relatively early timepoint.

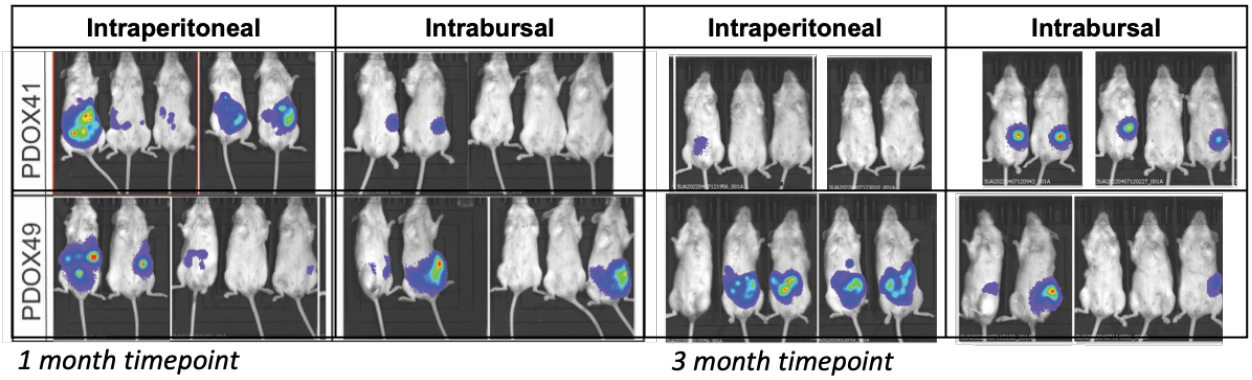
IB - cryopreserved tissue			
#52	n=5	Not known*	6 months
#55	n=5	Not known*	6 months

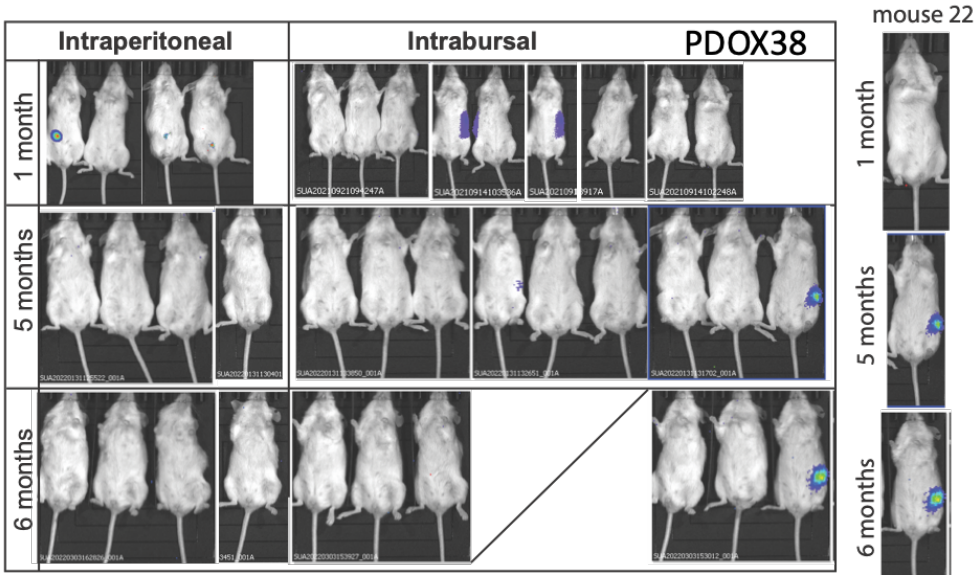
* Unclear if tumour is growing as no luciferase signal to check. Could be small tumours or none. Mice will begin to be sacrificed monthly starting from 9 months for autopsy analysis.

Conclusion: Although it is still early, we have ruled out the SC route as none have been successful. The IB route with luciferase-transduced organoids may be the most promising. Early luciferase signal is not a reliable indicator of tumour growth.

Delays to this aim were caused initially by the COVID-19 pandemic (closure of animal facility for new experiments in 2020) and secondly by the decision to employ luciferase-transduced organoids in an effort to obtain trackable intrabursal and intraperitoneal (= orthotopic) models. In the future we will continue to transduce the remaining 4 successful MOC organoids and test for in vivo growth these via IP and IB routes. Growing tumours will be harvested for cryopreservation and future propagation in mice.

Figure 7. Luciferase in vivo imaging of PDX attempts. For #41 you can see how the luciferase signal has decreased from 1 to 3 months in the IP, but may have increased in the IB. For #49 the signal may have decreased in some mice and increased in others. For #38 the early signals IP and IB at 1 month were mostly gone by 6 months, however, 1 mouse (22) had no signal at 1 month but an increasing signal at 5 and 6 months.





5. Major tasks 5 and 6. Characterisation of models

To undertake genomic and immunohistochemical profiling of successful models and compare to the primary tumor.

Methods: Tumour tissues and organoids were fixed and embedded and used for histology - H&E stain (morphology) and immunohistochemistry (IHC) of CK7, CK20, PAX8 (diagnostic markers), PAS (for mucin), p53 and HER2 (mutation markers).

DNA and RNA were extracted from tumours, organoid and normal lymphocytes (when available) and analysed by whole genome sequencing (when DNA was sufficient) or whole exome (when DNA was limiting). We had initial difficulties with the organoid DNA, which frequently failed sequencing QC if the amount submitted was limiting. It appeared there was some inhibiting substance that was present in the extracted DNA. When we Chelex-treated the DNA, this was alleviated and sequencing could be completed. However, some samples did not have enough DNA for sequencing after this clean-up process and had to be reextracted from another passage, which led to some delays.

Another problem encountered was that our frozen tissues often had poor morphology, making it hard to estimate the tumour proportion or to microdissect. This was not something encountered previously and may be a consequence of the tissue sitting in collection media and being quite wet when snap frozen. Increased water content could lead to greater freezing artifacts. Recently we have attempted to alleviate this issue by blotting excess liquid prior to freezing and embedding in optimal cutting medium (OCT). For

other tissues we have instead extracted DNA from the FFPE blocks which retain excellent morphology, although in 1 case there was insufficient cells in the FFPE block. This work-around leads to a slight drop in DNA quality and makes identifying structural variants more difficult but overall copy number and mutation are still able to be readily detected.

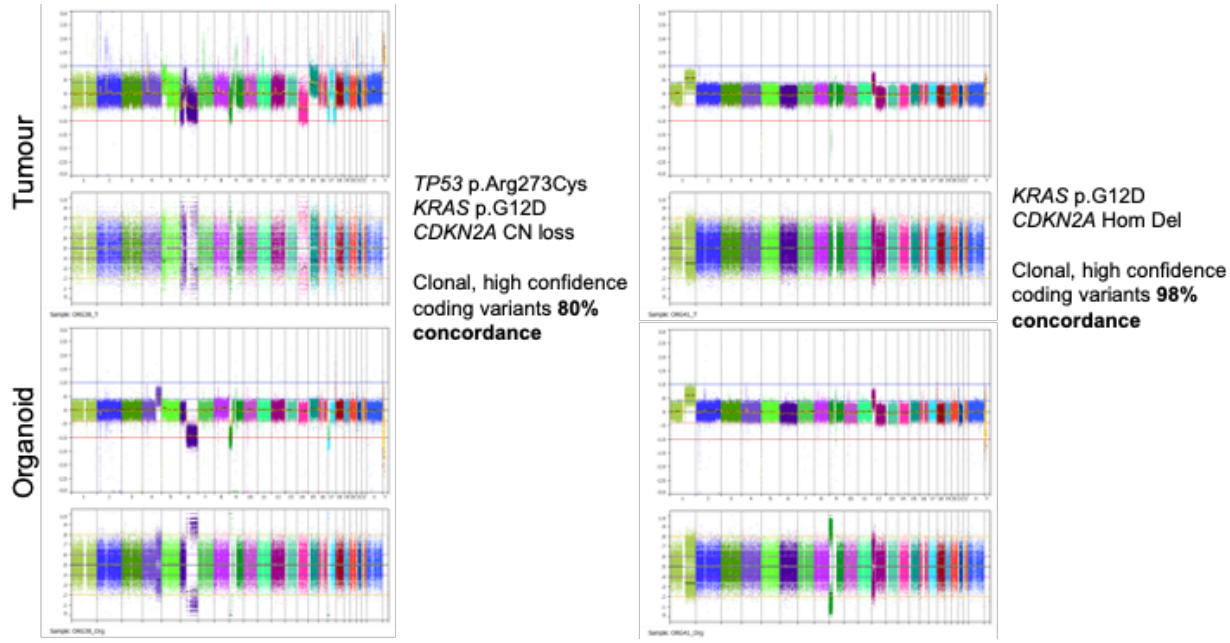
Results: Only organoid models have been sufficiently successful for full characterisation, cell lines and PDX will be performed in the future (if successful). Progress on these tasks indicated in Table 4.

Table 4. DNA was successfully extracted from all but 2 organoid lines. We have focused on sending MOC cases for sequencing; of these 3 have had data back (2 WGS), 4 are in progress, and in 1 case there was no tumour in the frozen and insufficient in the FFPE piece (*). OPAL and STR sequencing will be done when the cohort is complete.

Tissue ID#	Type	DNA T	DNA O	DNA seq T/O	IHC T	IHC O
27	Borderline	N	Y	N	Y	Y
42	Borderline	N	Y	N	Y	Y
44	Borderline	N	Y	O only	N (insufficient tissue)	Y
47	Borderline	N	Y	N	Y	Y
57	Borderline	In progress	In progress	N	In progress	In progress
38	MOC	Y	Y	Y	Y	Y
41	MOC	Y	Y	Y	Y	Y
26	MOC	Y	Y	Y	Y	Y
46	MOC	Y	Y	In progress	Y	Y
49*	MOC	N	N	N	Y	N
59	MOC	Y	Y	In progress	In progress	In progress
60	MOC	Y	Y	In progress	In progress	In progress
62	MOC	Y	Y	In progress	In progress	In progress

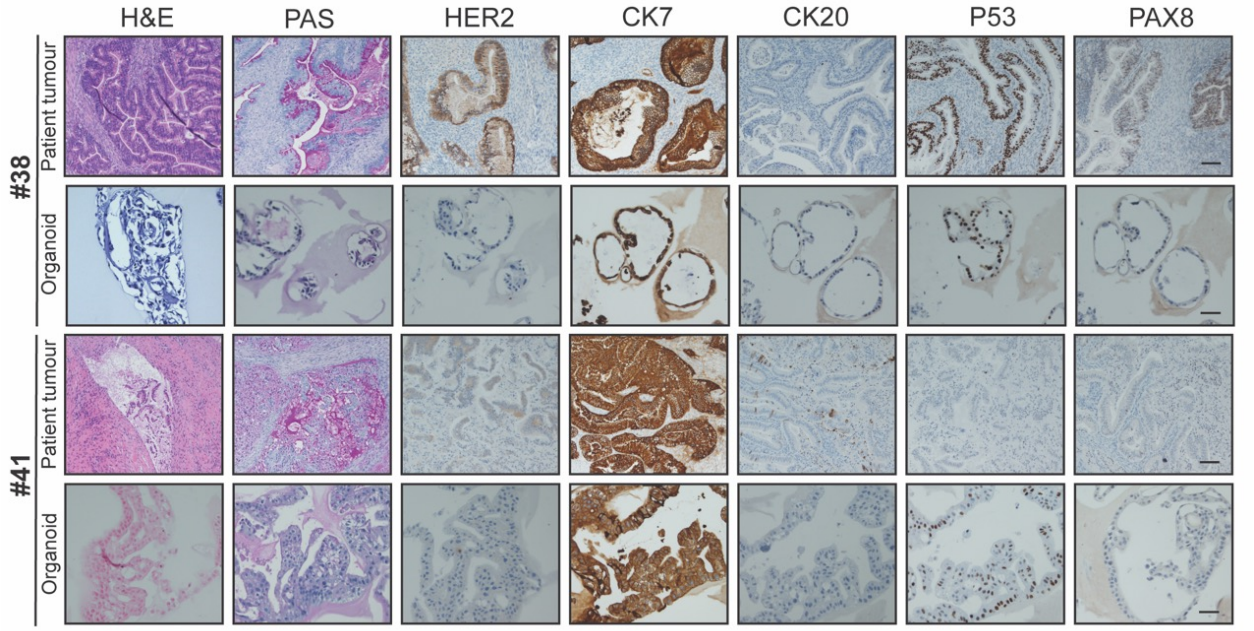
For samples analysed to date, there has been good to excellent concordance of copy number and mutation (Figure 8). #41 for example showed very high concordance of copy number and mutations. In contrast, #38 showed high concordance of key driver mutations (in *TP53*, *KRAS* and *CDKN2A*), but 80% concordance for mutations overall, and there was some variation in copy number. This was interesting because ORG38 had some morphological variation as well, with some organoids having a "light" cystic morphology, while others were "dark" and solid. We attempted to separate out organoids with these morphologies and performed RNAseq, but no strong gene expression changes were observed, even when we undertook a single-cell RNA sequencing pilot (funded elsewhere). In addition, the light and dark morphs did not appear to have different copy number events when we inferred this using the RNAseq data. This light vs dark morphology is something we are interested in exploring further: we have observed similar heterogeneity in some other organoid lines and it is not uncommon for MOC tumours to have histological intra-tumour heterogeneity.

Figure 8. Whole genome sequencing of two organoid models compared to the primary tumour. Shown are the copy number and B-allele frequency plots for #38 (left) and #41 (right).



IHC is completed for all with available tissue/organoids for CK7, CK20, PAX8, p53, Ki67 and ERBB2/HER2, with strong concordance between tumour and organoid lines (Figure 9).

Figure 9. Examples of IHC characterisation of two organoid cultures compared to the primary tumour



Conclusions: We have made good progress towards characterising the organoid models and this will be completed soon. Overall there is good concordance between organoids and tumours, with some differences that may be a consequence of tumour heterogeneity. We have started writing a manuscript describing the organoid models in particular.

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

A graduate research student (Masters of Biomedical Science, Olivia Craig) and a PhD student (Carolina Salazar) were 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 postdocs (Genevieve Dall and then Suad Abdirahman) working on the project have undertaken training to learn new PDX techniques.

Over the course of the project, all students and the PI attended weekly institutional seminars. In addition, the following professional development activities were undertaken:

Carolina Salazar:

- EMBL student Symposium in Sydney. Highly commended oral presentation award.
- EMBL cancer genomics conference. (Poster Presentation, Germany, 2019)
- Good-bye flat Biology conference. (Poster presentation, Germany, 2019)
- CRG Symposium. (Attendee, Spain, 2019)
- Patient-derived in-vitro models: Advances and challenges Symposium organised by The University of South Australia (Oral presentation, online, 2020)
- Australian New Zealand Gynaecological Oncology Group (ANZGOG) Annual Scientific meeting. (Oral presentation, online, 2021)

Olivia Craig:

- Canadian Conference of Ovarian Cancer Research poster presentation (online, 2021)
- Australian New Zealand Gynaecological Oncology Group (ANZGOG) Annual Scientific meeting 2021, poster presentation (online)
- Lorne Cancer Conference poster presentation (VIC, Australia, 2022)

Suad Abdirahman:

- Canadian Conference of Ovarian Cancer Research, poster presentation (online 2021)
- Lorne Cancer Conference poster presentation, poster prize (VIC, Australia, 2022)

PI Kylie Gorringe:

- Peter Mac Mentoring program, mentor Helen Hovenga (Head, People and Culture), 2019-20
- Peter Mac 360 Feedback Program, 2019
- Peter Mac "Scientists in the Clinic" Program 2019
- International Gynaecological Cancer Society Annual meeting, Rio de Janeiro (2019)
- Participation in Ovarian Cancer Australia National Action Plan Summit 2019
- ANZGOG Annual Scientific meeting, oral presentations 2019, 2022
- "Applied Good Clinical Practice Training" updated 2020
- GCIG committee attendee 2020-21
- Organising committee, Penny Taylor Oration (2021)
- Ovarian Cancer Australia Scientific and Clinical Expert Advisory Panel (2021-present)

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 spoke at a fundraising event at Peter MacCallum Cancer Centre (2021)
- The PI also presented an update at the Victorian Comprehensive Cancer Centre Gynae Stream Research Meeting (clinicians and researchers), and presented aspects of the work during invited seminars at 4 Australian and 3 international institutes.

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

Nothing to report (final report)

4. IMPACT:

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

We have developed the methods to be able to reliably grow cells from mucinous ovarian tumours in the laboratory, using a system where the cells are supported in 3D in a more physiological environment. We expect that our methods will be used by others to also grow similar cancer cell models, and indeed have already shared our protocols with two other ovarian cancer labs. However, we plan to publish in a peer reviewed journal to disseminate the results more widely. In the future, we will be using these new cell models of cancer to test therapies, for example new small molecules that target cancer-specific mutations and pathways. This is an exciting application because we hope to be able to direct clinicians towards more effective therapies for women with this disease, which is lethal when it spreads beyond the ovary.
In addition, we have made good headway in transferring these models into a mouse model. There are very few mouse models for mucinous ovarian cancer, but it is very helpful to be able to test therapies in a whole animal. In particular, if we develop new therapies that have never been used in humans before, we will need to evaluate them in an animal model such as the mouse before a human can be treated.

What was the impact on other disciplines?

Our methods are highly cancer-specific, therefore the impact beyond the field is likely to be minimal.

What was the impact on technology transfer?

Our methods have been supplied to other academic research laboratories. In the future, we will be able to share the methods and models themselves with researchers working in the field of ovarian cancer research for their own experimentation, which may include industry partners. These other researchers will be able to use our new models to also make exciting discoveries in ovarian cancer.

What was the impact on society beyond science and technology?

Ultimately we hope that our new model systems will change medical practice for mucinous ovarian cancer, when we use them to discover new medicines to treat women with this disease.

5. CHANGES/PROBLEMS:

Experimental/logistical issues encountered have been described above, such as changes to collection criteria, challenges in generating the various models, difficulties in DNA extraction etc.

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

The delay in funds being approved for use at the host institution and the delay in human ethics approval from HRPO led to significant delays in being able to start experiments.

Continued COVID-19 outbreaks 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, we were able to continue recruitment when restrictions eased.

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

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**

Journal publications.

Nothing to report specific to the experimental work under this award as yet, however a related review paper by the team was published:
Craig O, Salazar C, **Gorringe KL**. (2021) Options for the treatment of mucinous ovarian carcinoma *Current Treatment Options in Oncology*. 22(12):114. doi: 10.1007/s11864-021-00904-6. Invited review.

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

Craig O, Nigam A, Dall GV, **Gorringe KL** (2021) Rare Epithelial Ovarian Cancers: Low Grade Serous and Mucinous Carcinomas. In Perspectives in Medicine: Ovarian Cancer. Ed. Cold Spring Harbour Laboratories. accepted for publication 18th September 2020; doi: 10.1101/cshperspect.a038190

Other publications, conference papers and presentations.

Invited seminars:

The CI presented the work to the Victorian Comprehensive Cancer Centre Molecular Tumour Board and VCCC Gynae Research Group, as well as invited presentations at 6 institutes in Australia and 3 in the UK.

National conferences (team members):

- Australian and New Zealand Gynaecologic Oncology Group's Annual Scientific Meeting oral (2019, 2021, 2022), poster (2021)
- EMBL student Symposium, Sydney. Highly commended oral presentation award (2019).
- Patient-derived in-vitro models: Advances and challenges Symposium organised by The University of South Australia (Oral presentation, online, 2020)
- Ovarian Cancer Australia Symposium (2021)
- Lorne Cancer Conference, poster presentation prize (VIC, Australia, 2022)

International conferences (team members):

- EMBL cancer genomics conference. (Poster Presentation, Germany, 2019)
- Good-bye flat Biology conference. (Poster presentation, Germany, 2019)
- CRG Symposium. (Attendee, Spain, 2019)
- Canadian Conference of Ovarian Cancer Research, 2 poster presentations (online, 2021)

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

Nothing to report

- **Technologies or techniques**

Our methods of organoid generation and passaging have been shared with two research laboratories to date. Ultimately we will publish our methods for others to use.

- **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). We have generated 5 borderline and 8 MOC organoid lines to date. These will be available on request to share with other researchers, as will cell lines and PDX when successful. DNA sequencing data will be made available to others when published in the literature.

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:	6
Contribution to Project:	A/Prof Gorringe has supervised the project overall
Funding Support:	New Funding support: 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.

Name:	Dr Suad Abdirahman
Project Role:	Postdoctoral Scientist
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	17
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:	18
Contribution to Project:	Ms Craig has assisted with sample processing and culturing of organoids
Funding Support:	Australian Post-graduate award (2021-24) (PhD scholarship funding)

Name:	Dr Carolina Salazar
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	24
Contribution to Project:	Dr Salazar performed sample processing and culturing of organoids and was the person who initially optimised organoid culture. Her PhD was awarded in 2021.
Funding Support:	No change

Name:	Dr Genevieve Dall
Project Role:	Postdoctoral Scientist
Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0001-6205-7342
Nearest person month worked:	9
Contribution to Project:	Dr Dall developed a REDCap database to collate pathology information on samples to be received into the program and their laboratory uses (e.g. grown as PDX, snap frozen for DNA/RNA extraction etc). Dr Dall has also spent a portion of her time based in the Scott laboratory

	learning the animal skills and logistics required for generating PDX models. She obtained a research fellowship to work with Prof Scott and left the project.
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: -NHMRC Investigator grant (2022-2026) no overlap -CCV Venture Grant 2020-2021, no overlap) -Australian Cancer Research Foundation Infrastructure grant (AU$3.5 million, 2020-24, no overlap) Ovarian Cancer Research Foundation (2019 –2021) AUD, 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:	New Funding support: Tour de Cure: Senior Researcher Award AU$200,000 (no overlap)

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

Nothing to report

What other organizations were involved as partners?

Walter and Eliza Hall Institute (WEHI), Melbourne, Australia
Collaboration.
Co-I Prof Scott's lab is located at WEHI. Both Dr Abdirahman and Dr Dall visited the institute to receive training in mouse PDX procedures.

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES:

- *Award Chart*
- *Award Expiration Transition Plan*

W81XWH1810226 : Developing Clinically Relevant Models of Mucinous Ovarian Carcinoma for Testing Therapies

PI: A/Prof Kylie Gorringe, The University of Melbourne, Australia

Budget: $267,890.98

Topic Area: Ovarian Cancer Research Program

Mechanism: Pilot Award

Research Area(s): 1303, 1307, 0899

Award Status: 16 Sept 2018 – 15 April 2022

Study Goals:

To develop robust methods to reliably generate patient-relevant models of MOC for pre-clinical testing of existing and novel therapeutics.

Specific Aims:

Aim 1. To determine the optimal conditions under which MOC and borderline tumors can be cultured as primary organoids
Aim 2. To determine the optimal conditions under which MOC can be cultured as stable long-term cell lines
Aim 3. To determine the optimal conditions under which MOC can be cultured as patient-derived xenografts (PDX)
Aim 4. To undertake genomic and immunohistochemical (IHC) profiling of successful models and compare to the primary tumor.

Key Accomplishments and Outcomes:

- Successfully determined optimal conditions for organoid growth from MOC and borderline tumours. 8 MOC and 5 borderline long term cultures established (Aim 1 complete)
- Partial success in determining optimal conditions for culturing long term MOC cell lines: 1/7 successful to >p5; 2 others at p2-3.
- Partial success in determining optimal conditions for PDX: 2 definitely not successful (>6 months). 1 successful (tumour at 5 months); 2 ongoing (possible tumours at 3 months). Determined that subcutaneous route is not a good method, whereas intra-bursal route shows promise
- Success in IHC and genomic profiling of organoid models showing high concordance of genetic events and protein expression

Publications: none to date

Patents: none to date

Funding Obtained: OCRP Teal Expansion Award (2021-24) OC2000056

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product. ***This is not an application for funding; however, answers will help us understand the outcomes and products from your award.***

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** [X] or **No** [ ]
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?

MOC can be readily grown as organoids as long term models with the optimal media, with genomic and histopathological features consistent with the primary tumours. Cell lines are difficult to establish from primary tissue using conventional media due to overgrowth by fibroblasts, however we had success in going from organoid cultures to 2D cell lines using OCMI medium. MOC do not grow well in mice at a subcutaneous site, however intrabursal transplantation of cells grown from organoids had the most success and this may be a better method, as well as being orthotopic.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award*.

We have been awarded funding from the DoD Ovarian Cancer Program via the TEAL Expansion award.
Total budget
Title "Testing therapies for mucinous ovarian cancer in novel patient-derived models"

4. What will be **the next step(s)** for this project?

Firstly, to continue to expand the organoid, cell line and PDX models bank of MOC, finalising methods and characterisation for the latter two model formats.
Secondly, to use these models to test existing and new therapies using the new funding described above.
Finally, we are exploring ideas of co-culturing the organoid lines with fibroblast cultures generated as a by-product of unsuccessful MOC cell line attempts. The stromal microenvironment could be important in mediating chemotherapy resistance and promoting a more aggressive subtype of MOC.

5. How would you classify your **lead candidate product**? *Please choose the best option or add explanation for multiple selections.*
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Please choose, if applicable
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?

Generation of patient-derived models for MOC will enable future research to determine the best therapy for this disease, which will impact women in all the above categories.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.

The current product is considered to be a research tool that is not readily amenable to commercialisation. However, we are able to share our models with the research community as well as our methods so that others can generate their own models from cancer tissue from this disease. There is potential that our organoid methods could be used as a method for personalised therapy selection, however the knowledge we have generated is based on research by others without clear IP.