AWARD NUMBER: W81XWH-18-1-0226

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

PRINCIPAL INVESTIGATOR: Associate Professor Kylie Gorringe

CONTRACTING ORGANIZATION: Peter MacCallum Cancer Centre
Parkville, VIC 3010, 3000 AU

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Associate Professor Kylie Gorringe

E-Mail: kylie.gorringe@petermac.org

5d. PROJECT NUMBER

5e. TASK NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Peter MacCallum Cancer Centre 305 Grattan Street, Melbourne, Victoria, Australia, 3000

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U.S. Army Medical Research and Materiel Command
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14. ABSTRACT

This project aims to develop patient-derived laboratory models for mucinous ovarian cancer. During the course of the reporting period detailed herein, a human ethics amendment at the host institution was approved (Appendix 1) and animal ethics has been obtained (Appendix 2) to cover all experimental procedures proposed in this grant. Significant delays in funding were experienced due to contract negotiations however a postdoctoral scientist with extensive experience in animal models and tumour biology was recruited to the project on the 1st of July 2019. Dr Dall has begun training under the supervision of Professor Scott in preparation for biospecimen collection and processing.

15. SUBJECT TERMS
Human ethics, mouse ethics, personnel recruitment

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Contents

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. OVERALL PROJECT SUMMARY

This project aims to develop and validate clinically relevant models of mucinous ovarian cancer with the view that such models will allow for greater advances in treatment discoveries. We plan to recruit to our study 10 mucinous ovarian carcinoma samples and 10 mucinous ovarian borderline samples over the next two years. With these samples we will optimize conditions for culturing these samples in 2D and 3D *in vitro* growth and as *in vivo* patient-derived xenografts (PDX). 2D cell lines derived from tissue samples are the easiest and cheapest model we can create. They serve as long-term, shareable resources that have many applications. Screening for new drug treatments can be carried out in very high throughput using cell lines and genetic manipulation to identify cancer driving mutations are more straightforward. The limitation of 2D cell lines is that they do not take into account 3D growth considerations. This can be overcome by culturing the cells as 3D organoids. Organoid culture is less economical than 2D culture due to the expensive reagents required, but they more accurately represent the tumor as they allow for more physiologically relevant cellular orientations, whilst still providing an easily controllable experimental system. Although PDX models come with their own share of limitations (cost, variability from mouse to mouse), they are the most clinically relevant model researchers have. PDX models allow for clinically equivalent treatment modalities (surgical excision, intravenous dosing), innervation of vasculature and require minimal disruption to the integrity of the tumour tissue.

By creating and validating (via genomic and immunohistochemical profiling) models of mucinous ovarian cancer that cover all three of these *in vitro* and *in vivo* model types we are allowing for the greatest potential in clinical improvement for mucinous ovarian cancer sufferers.

4. KEY RESEARCH 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	0
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	0
Subtask 2: Obtain fresh tissue from second 10 patients and test refined set of conditions based on the outcome of Subtask 1.	10-20	0
Subtask 3: Characterize successful organoids for morphology and growth	13-24	0

rates		
Milestone#1 Establish the optimal conditions for growth of MOC as organoids	20-24	0
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	0
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. • 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	0
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 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	0
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	0
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	0
Major Task 6: Characterization of successful models		
Subtask 1: Extract DNA from successful cell lines, organoids and PDX tissue	18-20	0
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	0
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	0
Subtask 6: Analysis of whole genome sequencing data	22-24	0
Milestone#4 Author manuscript(s) on the optimal conditions for development of MOC models and describe the successful models in detail.		

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

As shown in the table above (SOW), in this reporting period, two tasks were due for completion:

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

Relevant Human Ethics documents were first submitted online in June 2018. The first communication from HRPO was January 2019. Since then we have updated our local HREC protocol as requested (approval obtained 19th June 2019 (Appendix 1)), and provided training documents and annual reports (intermittently as requested over this period). The last requested documents were submitted 29th August and we now await further communication. As per the stipulations of our human ethics we cannot collect human tissue into the laboratory until these approvals are obtained. The remaining tasks cannot therefore be undertaken until HRPO approval is provided.

2. Specific Aim 3: Subtask 1: Submit documents for Animal Ethics review. An application for animal ethics pertaining to the proposed animal experiments in this grant was submitted to the Peter MacCallum Cancer Experimentation Ethics Committee (AEEC) and was approved 1st November 2018 (Appendix 2). This approved application was forwarded to the DoDs ACURO and approved on 13th February 2019.

A postdoctoral researcher with 8 years' experience in animal handling and tumour modelling was recruited in July 2019. Dr Dall has been included as an additional investigator on the Peter MacCallum Cancer Centre Animal Ethics. Dr Dall has also set up a REDCap database to enter in pathology data from patient samples that will be received into the laboratory. This database will also track the application of the tissues in the laboratory and allow for accurate record keeping, assisting all present and future laboratory members using these resources.

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

Dr Dall has spent time in Co-CI Professor Scott's laboratory learning the techniques used by the Stafford Rare Fox Program researchers to grow human tissue as cell lines, organoids and in mice as PDX.

- **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?**

In the next reporting period we expect to be granted all ethics approvals to allow for the collection of human tissue. This will allow us to begin to test the conditions for growing mucinous ovarian tumour tissue as cell lines, organoids and PDX models as described in specific aims 1, 2 and 3 above. Human ethics approvals will also allow for the collection of frozen and formalin-fixed tissue to begin the tasks specified under Aim 4.

5. 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

6. 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**

There was a significant delay in receiving funds for this grant due to the contract negotiations. As a result, an internal cost centre was only set up in June 2019. To ensure this delay in funding does not significantly hinder progress on the project, a postdoctoral researcher with extensive experience in animal handling, mouse surgeries, and tumour measuring was recruited to the project. Consequently, minimal training is required and as soon as human ethics is approved by HRPO, tissue collection and processing will be initiated. We have also scoped out required reagents and materials and have begun ordering these in so we can begin experiments promptly on approval.

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

The delay in funds being approved for use at the host institution has meant that expenditures for year 1 have also subsequently been delayed. A postdoctoral researcher was not recruited to the project until July 1st 2019. Laboratory experiments have not yet begun as we are still awaiting 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

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

Nothing to report

7. PRODUCTS:

○ 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

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).

8. 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 animal ethics application for approval by Peter MacCallum Cancer Centre's AEEC which was approved on 1st November 2018 (Appendix 2). A/Prof Gorringe recruited Dr Dall and added Dr Dall as additional investigator on animal ethics (Appendix 3).
Funding Support:	n/a

Name:	Dr Genevieve Dall
Project Role:	Postdoctoral Scientist
Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0001-6205-7342
Nearest person month	3

worked:	
Contribution to Project:	Dr Dall has 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.
Funding Support:	n/a

Name:	Professor Clare Scott
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0002-3689-5956
Nearest person month worked:	3
Contribution to Project:	Intellectual contribution and training for Dr Dall in the generation of PDX models.
Funding Support:	Update to previously reported funding: Previously Active now Completed None New funding support **Title**: Targeting G9a methyltransferase to block metastasis and overcoming chemotherapy resistance. **Funding Agency:** Ovarian Cancer Research Foundation **Funding Period:** 2019 –2021 **Funding amount**: $190,000 AUD **Role:** CIB; 5% **Project goals and specific aims:** This project aims to validate G9a and its target genes as predictive markers of metastatic recurrence and to determine whether G9a inhibition is efficacious in treating chemoresistant PDXs. Aim 1: Validation of G9a and its target genes as predictive markers of metastatic recurrence using ovarian tumours. Aim 2: Determine the efficacy of G9a inhibitor inducing cell death in chemoresistant ovarian PDXs. Aim 3: Determine the efficacy of G9a inhibitor as an agent to improve response to immunotherapy. Aim 4: Determine the mechanism by which G9a inhibitor acts as an anti-cancer agent **Overlap:** None as this involves a study on the effectiveness of G9a inhibitor in ovarian cancer PDX models.

Name:	Professor Rob Ramsay
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	ORCID ID: 0000-0001-5003-0433
Nearest person month worked:	3
Contribution to Project:	Intellectual
Funding Support:	Update to previously reported funding:

	Previously Active now Completed **Title:** Deciphering the overlapping roles of SSB1 and SSB2 in the regulation of haematopoiesis and intestinal homeostasis **Time commitments:** 5% **Supporting agency:** Australian National Health and Medical Research Council **Period:** 2015-2018 **Funding:** $958,728 **Aims:** Our work centres on elucidating the role of two newly identified and related single-stranded DNA binding protein (Ssb1 and Ssb2) in development of blood and gut system. When both genes are deleted mice die with 8 days of knockdown due to bone marrow failure and intestinal atrophy. Our double knockout model parallels the consequences of radiation damage on blood and gut system. Toxicity to these systems is a significant hindrance in delivering anti-tumor therapy. **Overlap:** None **Title:** Vaccination against Adenoid cystic and Colorectal Carcinoma Using MYB cDNA - VACCUMeD Clinical Trial - Immune Modulatory Therapy in Colorectal and Adenoid Cystic Carcinoma **Time commitments:** 10% **Supporting agency:** Victorian Cancer Agency **Period:** 2016-18 **Funding:** AU$1,343,655 **Aims:** The tumour cells in patients with bowel cancer and certain head and neck cancer harbour a particular factor that orchestrates multiple pro-cancer processes. We have found that the aberrant production of this factor allows the specific targeting of these cancers by a novel vaccine. Using this vaccine in combination with the blockade of the recently discovered immune system check-point regulators we have developed robust pre-clinical evidence for symptom-free cancer control and cures. In this new project we aim to advance the vaccine into patients to test its safety and as a secondary aim to monitor for evidence of vaccine efficacy. If successful, this specific vaccine has broad applicability and more widely the concept is readily transferable to other cancers. **Overlap:** None New funding support None

- **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?**

Nothing to Report

9. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**

Nothing to report

- **QUAD CHARTS:**

Nothing to report

10. APPENDICES:

Peter MacCallum Cancer Centre
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Melbourne Victoria
3000 Australia

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Locked Bag 1 A'Beckett Street
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Locations
Melbourne
Bendigo
Box Hill
Moorabbin
Sunshine

Peter Mac
Peter MacCallum Cancer Centre
Victoria Australia

MEMORANDUM

To: Prof Ian Campbell

From: Ethics Committee Secretariat

CC: Dr Kylie Gorringe

Date: 04 July 2017

Re: **Peter Mac Project No:** 14/76
Study Title: GAMuT (Genomic Analysis of Mucinous Tumours): Improving outcomes for patients diagnosed with mucinous cancer

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We are pleased to advise that the amendment submission dated 19 June 2017 has **received ethical approval** from the Peter MacCallum Cancer Centre Human Research Ethics Committee. The amendment also **satisfies Peter Mac Research Governance requirements** and may now be conducted at this site.

The following documents have been reviewed and approved:

Document	Date
Protocol-*GAMuT (Genomic Analysis of Mucinous Tumours): Improving outcomes for patients diagnosed with mucinous cancer*	19 June 2017

Should you have any questions about your project please contact the Ethics Committee Secretariat on (03) 8559 7540 or ethics@petermac.org. Submission guidelines, forms and standard operating procedures are available on the ethics website: http://www1.petermac.org/Ethics/default.htm

Kind Regards,

Robyn Summerhayes
Ethics Committee Secretariat

The Peter MacCallum Cancer Centre Human Research Ethics Committee and it's subcommittees are organised and operate in accordance with the National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Research Involving Humans (2007), and all subsequent updates, and in accordance with the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and the Health Privacy Principles described in the Health Records Act 2001 (Vic) and Section 95A of the Privacy Act 1988 (and subsequent Guidelines).

Please be advised that the Principal Researcher and Associate Researchers named on the application did not participate in deliberative discussions or decision-making regarding the project.

PETER MACCALLUM CANCER CENTRE ANIMAL EXPERIMENTATION ETHICS COMMITTEE (AEEC)	
Application For Approval To Use Animals In A Research Project	**Date application received: 31/05/18** **Resubmitted: 17/07/18; 15/09/18; 19/10/18; 31/10/18**

Office Use Only

Project Title AEEC Register Number

New patient-derived mouse models for ovarian cancer

AEEC Permit Number

	E	6	1	8

DECLARATION BY AEEC CHAIRMAN

I certify that this project has been considered and approved by the Peter MacCallum Cancer Centre AEEC Chair on the 1st November 2018 and ratified by the full AEEC on 6th December 2018.

The period of approval for this project is 01/11/18 to 31/10/21

AEEC Chairman Name:	**Prof Phillip Darcy**
AEEC Chairman Signature:	
Date:	**07/01/19**

CONDITIONS OF APPROVAL

All matters pertaining to the conduct of the approved project are to be reported to the AEEC, which maintains oversight in accordance with licence conditions for the Licence SPPL20183.

Any variation proposed to the project, and the reasons for that change, must be submitted to the AEEC for approval and must not be implemented until approval is granted.

A record of details of any animals used in the project must be retained.

The project should only be conducted in approved premises nominated on the Bureau of Animal Welfare Scientific Licence **SPPL20183.**

The AEEC must also be notified in writing of;

- Any changes to approved investigators
- Any unexpected incidents or complications that result in deaths, euthanasia or pain and suffering for the animals used in the project. Details of the steps taken to deal with adverse incidents must be included in the notification.

OTHER CONDITIONS:

This approval is subject to the following special conditions:

None.

APPLICATION FOR APPROVAL TO USE ANIMALS IN A RESEARCH PROJECT PETER MACCALLUM CANCER CENTRE

KEY RESPONSIBILITIES

All scientific procedures using animals must be carried out in accordance with the Prevention of Cruelty to Animals Act 1986 Act (the Act), associated Regulations and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (the Code).

These legislative requirements specify that an Animal Ethics Committee (AEC) must verify that the use of animals for research or teaching is justified and adheres to the principles of Replacement, Reduction and Refinement. All proposed animals use must be approved by an AEC before commencing the project.

Before completing this application form investigators should be familiar with the following as applicable:

- The Australian Code of Practice for the Care and use of Animals for Scientific Purposes (the Code).
- The Code of Practice for the Housing and Care of Laboratory mice, rats, guinea pigs and rabbits (the Housing Code)
- Part III of the Prevention of Cruelty to Animals Act 1986 and Regulations 2008 (the Act)

Knowledge of these legal requirements will assist you in completing this application in a satisfactory manner. The above documents can be found at www.dpi.vic.gov.au/animalwelfare/

NOTES ON THE COMPLETION OF THIS APPLICATION FORM

1 Insert your answers in the boxes provided below each question. When necessary boxes will expand to accommodate the length of your answer.

2 A response is required for each question. **Write "Not applicable", if necessary**.

3 **Applications must be written in plain English.** It should be assumed that assessors have either no scientific knowledge or no knowledge of your area of research. Where scientific language is unavoidable, it must be supported by a suitable lay description or a glossary of terms. **It is not appropriate to include sections from grant applications containing excessive detail of procedures unrelated to the use of animals.**

4 It is highly recommended that you ask a colleague and a person with a non-scientific background to read the application before it is submitted.

5 Where SOPs are available for a procedure, please refer to the SOP rather than describe the procedure.

SECTION 1 ADMINISTRATION

1.1 Title

The title of the project should be concise and expressed in lay language. Do not use abbreviations or scientific jargon.
New patient-derived mouse models for ovarian cancer

1.2 Principal Investigator

This will be the person who has legal responsibility for the welfare of all the animals being used.	
Name (Title, given name, family name)	**Dr Kylie Gorringe**
Are you employed by Peter Mac?	**Yes**
Main contact for this project (please note legal responsibility always remains with the Principal Investigator)	**Kylie Gorringe**

1.3 Duration of Project

Applicants may request approval for a project up to three (3) years. DO NOT nominate a date that is before the meeting of the AEEC.	
Proposed Start Date:	**01-11-18**
Expected End Date:	**01-11-21**

1.4 Animals Requested

NB: Embryos greater than half gestation (10.5 days) are counted as a whole animal.
Every strain requested for use must be listed here.

Species **(and common name)**	**Strain** **(Include background strain information)** * indicate with {*} genetically modified strains	**Sex**	**Age**	**Total Number**
e.g. Mice	*C57Bl/6: E6AP KO**	*m/f*	*6-8 wks*	*300*
Mus musculus (mouse)	Non-Obesity Diabetic SCID (NOD-SCID) mice lacking the Inter-leukin 2 gamma receptor (NSG)	f	6-10 wks	2440
Total number of all mice requested for project				**2440**

1.5 Funding

Is this project externally funded?		
☐	**No**	
☒	**Yes**	Please provide details US Department of Defense OCRF Pilot Study

1.6 Risk Management

Please identify risk areas associated with your application. Each identified risk area requires a risk assessment be undertaken and attached to this submission.	
☒	**Biohazard**
☐	**Radiation**
☒	**Chemical (including Cytotoxic drug)**
☐	**Other** (please provide details_____)

1.7 Permits

Is the acquisition, holding, or use of the animals/ organisms subject to any permit, law or regulation of the State or Commonwealth (eg. OGTR, protected native or imported)?		
☒	**No**	
X	**Yes**	If yes, please specify the permit number.

Organism / strain	Permit/dealing Number
NSG	**NLRD 8578.**

- *DEALING NUMBERS ARE REQUIRED FOR ALL DEALINGS WITH GENETICALLY MODIFIED ORGANISMS (E.G. MICE, VIRUSES ETC). PLEASE CONTACT THE PHYSICAL ENVIRONMENT AND INFRASTRUCTURE MANAGER OR THE OH&S PROJECT OFFICER FOR DETAILS.*

SECTION 2 PROJECT INFORMATION

The AEEC must be satisfied that the use of animals is justified, based on whether the scientific or educational value of the work outweighs the potential impact on the animals being used.

The Code emphasises the responsibilities of investigators, teachers and institutions using animals to:

- ensure that the use of animals is justified, taking into consideration the scientific or educational benefits and the potential effects on the welfare of the animals;
- ensure that the welfare of animals is always considered;
- promote the development and use techniques which replace animal use in scientific and teaching activities wherever possible;
- ensuring appropriate anaesthesia for the entire duration of an operation;
- minimise the number of animals used in projects; and
- avoid pain or distress for each animal used in scientific and teaching activities, including appropriate use of analgesia and painless euthanasia and killing of animals.

To this end, there is a need in scientific and teaching activities to consider:

- the *replacement* of animals with other methods
- the *reduction* in the number of animals used; and
- the *refinement* of techniques used to reduce the impact on animals

Overall, answers provided in the following sub-sections should provide AEEC members, particularly external lay and welfare members, with a clear idea of why the experiments are necessary and what happens to the animals.

All information provided in this section must be in language that can be understood by an interested, intelligent person without a scientific background.

PLEASE do not use scientific jargon and abbreviations or ensure terms are defined in the glossary below.

Glossary of terms
(please include acronyms)

Scientific Term	Lay Description
tumour organoid	growing tumour cells *in vitro* using a matrix that supports more physiological 3D growth
NOD SCID IL-2receptor gamma-/- mice	Non-Obesity Diabetic, Severe Combined Immunodeficiency mice lacking the Inter-leukin 2 gamma receptor: Mice which lack mature T cells, B cells. Serum antibodies are not detectable and natural killer (NK) cell cytotoxic activity is extremely low. Immunocompromised degree: severe.
Genetic/genomic	Related to genes.
in vitro	not in a live animal, e.g. in a culture dish
in vivo	in a live animal
non-high grade serous ovarian cancer	a group of ovarian cancer tumours that are not the most common subtype (high grade serous)
mucinous carcinoma	a subtype of ovarian carcinoma that produces mucin
necrotic	dead (usually referring to an area of tissue)
subcutaneous	under the skin

intra-muscular	inside the muscle
intra-peritoneal	inside the body cavity
Matrigel	a protein mixture that forms a gel substrate that supports cell growth

2.1 Project Summary

2.1.1 Provide a brief discussion of the background of the project. If applicable, describe how this project relates to any previously approved projects. (maximum 200 words)

This is a new project. We have previously studied human non-high-grade serous ovarian cancer using genetic approaches to discover new genes that could lead to new therapies. Recently we have begun to investigate possible therapies using *in vitro* systems (cell lines and tumour organoids). We now have funding to develop patient-derived xenograft models for mucinous ovarian cancer, and are seeking funding for the other rare subtypes. It will be important to be able to test new therapies *in vivo* because *in vitro* systems do not necessarily represent the patient situation. This may be particularly important for rare ovarian subtypes like mucinous carcinoma, which produce mucin *in vivo* that might prevent drug entry to the tumour. Such an effect may not be apparent with an *in vitro* system.

2.1.2 State the aim/s of the project (in bullet points)

- **Aim 1 Pilot study to evaluate methods of patient derived xenografts for ovarian cancer**
- **Aim 2 To develop new mouse models of ovarian cancer using patient tumour tissue**
- **Aim 3 To test drug therapies on these mouse models for efficacy**

2.2 Project Description

Please explain the scientific rationale of the project and provide a detailed description of the experimental design.

- Particular emphasis should be placed on describing what will happen to each animal or group of animals (including controls) from the time the animals are obtained until the time the project is completed. It is not necessary to include excessive detail about procedures not involving the use of live animals.
- When multiple procedures are to be performed on individual animals, consider using a flow diagram to illustrate the number of procedures to be performed and the time interval between each procedure. The expected effect on the animals of the procedures should be described.
- If performing a non-terminal surgical procedures describe how asepsis will be maintained during surgery and the pain management strategies that will be used to minimise post-surgical pain and distress.
- If agents are to be administered **provide details of dose rates, volumes, needle gauges, routes and methods of administration.** Also provide a brief description of the mechanism of action and expected effects of any agents to be administered.

Where scientific language is deemed unavoidable it must be supported by a suitable lay description in the text or in a glossary of terms.

Ovarian cancer is a lethal disease, with a low overall survival. It comes in several different subtypes, each of which is thought to derive from a different cell lineage (Figure 1). While the most common type, high-grade serous carcinoma, is well studied with multiple pre-clinical

models available, the other types are not well represented most likely due to their rarity (low-grade serous, mucinous, clear cell and endometrioid, collectively "non-high-grade serous"). These other subtypes are also more likely to be resistant to the standard ovarian chemotherapy combination (cisplatin/taxane). In order to better develop new therapies for these rare subtypes, we propose to develop additional pre-clinical models, including patient-derived xenografts (PDX).

We currently have funding to support the development of PDX models for mucinous carcinomas (MOC) and are seeking funding for the other subtypes. This proposal will therefore focus on MOC, but the methods are planned to be applied to the other subtypes.

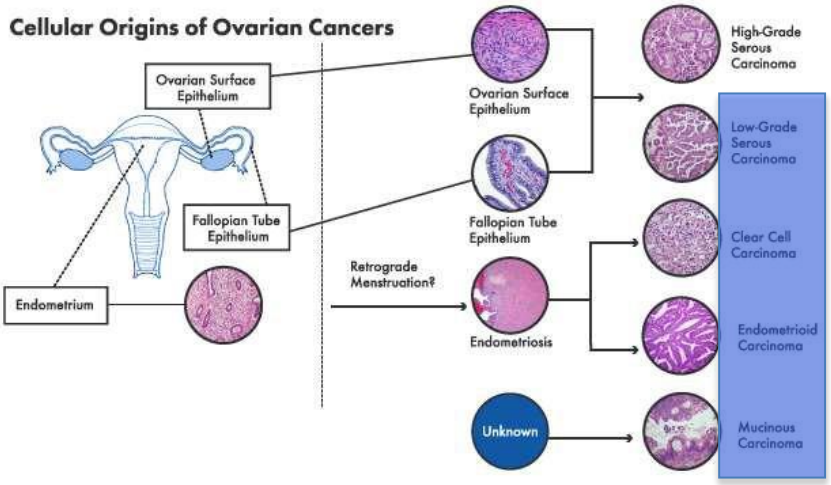


Figure 1. The subtypes of ovarian cancer and how they relate to the cell of origin. This study focussed on the non-high grade serous subtypes in the blue box. Source: http://resources.Nationalacademies.org/Infographics/OvarianCancers/images/Cancer-origins.png

Aim 1 Pilot study to evaluate methods of patient derived xenografts for ovarian cancer

Patient-derived xenografts (PDX) models are invaluable tools for evaluating the efficacy of cancer therapies in an *in vivo* situation. We will be evaluating several different methods for developing PDX models from these subtypes, beginning with mucinous ovarian carcinoma (MOC). Samples will be collected at surgery via the Australian Ovarian Cancer Study (AOCS) and other tissue banks and be evaluated by a pathologist to avoid necrotic areas. Human ethics approval has been obtained for MOC (HREC 14/76) and is being prepared for other tumour types.

At this stage we do not know which method of PDX generation will be the most successful, therefore we will undertake a two-stage strategy to optimise PDX generation for these tumours, beginning with a pilot study of 5 cases (for each subtype).

a) For the first 5 cases of each cancer type (e.g. MOC), we will test four conditions:

1) sub-cutaneous (SC) inoculation of dissociated tumour cells in Matrigel

2) sub-cutaneous implantation of a tumour piece

3) intra-peritoneal injection of dissociated tumour cells (IP)

4) Intra-muscular implantation of a tumour piece (IM)

Each method will be evaluated in 3-6 mice per case. The number will depend on the size of the tumour piece available. All animal surgery will be performed in a Category II Biohazard Cabinet to maintain sterility.

1) Sub-cutaneous (SC) injection of dissociated tumour cells in Matrigel

A tumour piece (1 cm x 0.5 cm x 0.5 cm) will be cut into small (2 mm) pieces and dissociated with the GentleMACs prior to mouse inoculation. For this procedure mice will be anaesthetised with isoflurane (**SOP 21.3.54**). 25-50µl of tumour cells (in PBS/50% Matrigel) containing 0.5-2x10^6 cells will be injected into each mouse into the right flank following **SOP 21.3.66.**

Following subcutaneous injection, mice will be caged with food and water available ad libitum in the warming cabinet for 1-3 h until they have recovered from anaesthesia. Researchers will monitor the mice during the recovery period until they are conscious and mobile.

In the rare event (<1 out of every 200 mice, <0.5% based on others past experience) that a mouse does not recover from anaesthesia (e.g. no signs of breathing, "bluish" skin, cold to touch) they will be removed from the container, have their neck dislocated and disposed of in yellow biological hazard waste bins. Any such occurrences will be recorded as an adverse event.

2) Sub-cutaneous implantation of a tumour piece

A 2x2 mm slice of tumour tissue will be placed in the right flank of each anesthetized mouse as per **SOP 21.3.73**, section 5.3.1. For postsurgical pain management, 5mg/kg Rimadyl (Carprofen) will be injected subcutaneously on the hind flank, daily for 3 days post-surgery (**SOP: 21.3.46**)

Following SC transplantation, mice will be caged with food and water available ad libitum in the warming cabinet (kept here for 3 hours to overnight) until they have recovered from anaesthesia. Researchers will monitor the mice during the recovery period until they are conscious and mobile. Researchers will continue to monitor the mice on the two days following surgery. In particular we will look for signs of distress (pain) and abnormal behaviour such as hunching, lack of grooming, bleeding from surgical wound, lethargy or hyperactivity, and abnormal gait. If signs of distress are evident then researchers will liaise with the experienced animal house staff to determine an appropriate course of action. Interventions such as providing ensure/nectar or placing animals on a heat mat will first be employed, and the animal carefully monitored. If the distress is not alleviated then mice will be euthanized by cervical dislocation (**SOP 21.3.06**) or CO_2 inhalation (**SOP 21.3.04**).

3) Intra-peritoneal injection of dissociated tumour cells (IP)

A tumor piece (1 cm x 0.5 cm x 0.5 cm) will be cut into small (2 mm) pieces and dissociated with the GentleMACs prior to mouse inoculation with 0.5-2x10^6 cells per mouse following **SOP 21.3.65**. Cells will be injected on the right side of each anesthetized mouse using an 26G needle. For this procedure mice will be anaesthetised with isoflurane (**SOP 21.3.54**) and monitored afterwards as for SC injection.

Mice exhibiting signs of distress, including lethargy and/or hunched appearance will be humanely euthanized using cervical dislocation (**SOP 21.3.06**) or CO_2 inhalation (**SOP 21.3.04**). Monitoring will also include regular observations of weight and abdominal distension (every 2-3 days or daily if weight is close to a reduction of 20%). Mice will be euthanized if weight loss exceeds 20% or if abdominal distension is considered to be due to significant ascites burden (> 2 ml), or earlier if signs of distress are evident.

4) Intra-muscular implantation of a tumour piece (IM)

A 2x2 mm slice of tumour tissue will be placed in the dorsal muscle of each anesthetized mouse as per **SOP 21.3.73**, section 5.3.2 and monitored as in 2) above.

b) Serial passaging of successful xenografts to show that they can be maintained

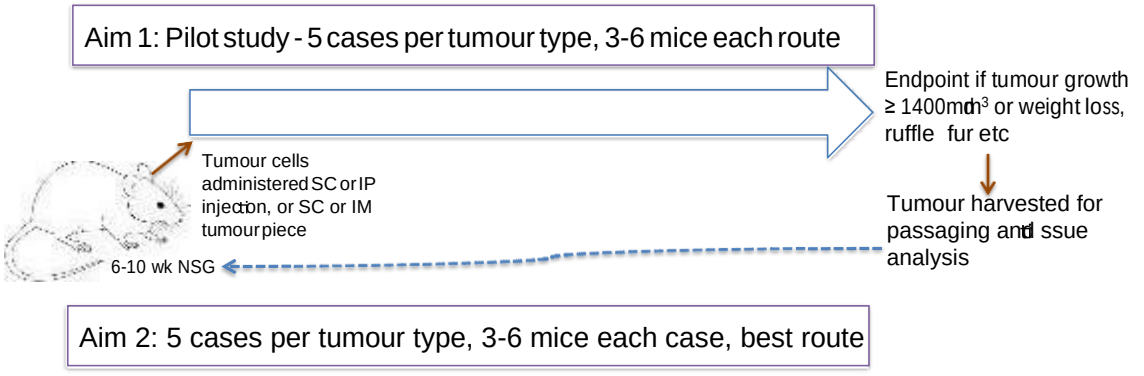
Once we have established the best method for growing primary xenografts we will passage the xenografts through NSG mice in order to retain the inherent biological features of the original tumour. Once they reach maximal size, as measured by electronic callipers (≥1400mm^3) the mice will be anaesthetized with isoflurane (**SOP 21.3.54**) and the tumour tissue surgically removed. This tumour tissue will be processed and placed in new mice using the best method determined above. Mice will subsequently be euthanized by CO_2 inhalation (**SOP 21.3.04**) or cervical dislocation (**SOP 21.3.06**). Removal of the xenograft prior to euthanasia will ensure that the biological features and viability of the human tumour xenografts are not compromised prior to removal. Tumour fragments will also be frozen down for long-term storage in DMSO for future transplantation.

Mice that do not develop signs of tumour growth will be monitored until 18 months old before euthanizing by CO_2 inhalation (**SOP 21.3.04**) or cervical dislocation (**SOP 21.3.06**).

c) Adaptation of in vitro models to in vivo

If none of the above methods are successful from primary patient material, we will attempt to create an *in vivo* model using cell lines or tumour organoid models being developed concurrently

from the same patient material. These will be attempted using methods 1) and 3) as described above using 3-6 mice per case.



Aim 2 To develop new mouse models of ovarian cancer using patient tumour tissue

We will evaluate the success rate of the four methods tested in Aim 1. If there is a method that is superior, this will be used in the second 5 cases. If methods are equally successful, we will prioritise as follows: IP > IM > SC/tumour piece > SC/matrigel. IP will most closely mimic the conditions in which these tumours grow in humans, while using a tumour piece is methodologically less work than generating single cell suspensions. We will apply the best method to subsequent cases on a tumour-type specific basis.

Aim 3 To test drug therapies on these mouse models for efficacy

After PDX have been established, they can be used for testing therapies.

a) Drug tolerability. For drugs that have been previously tested at Peter MacCallum Cancer Centre in NSG mice, we will use a recommended tolerable dose. The therapies to be used are mostly characterised and have been used in mouse studies previously as most are either in practice or clinical trials, therefore we expect most to be in this category (e.g. tamoxifen, trastuzumab, APR-246). We do not know all of the drugs that will be used as some will depend on the outcome of genomic analysis of the patient tissue.

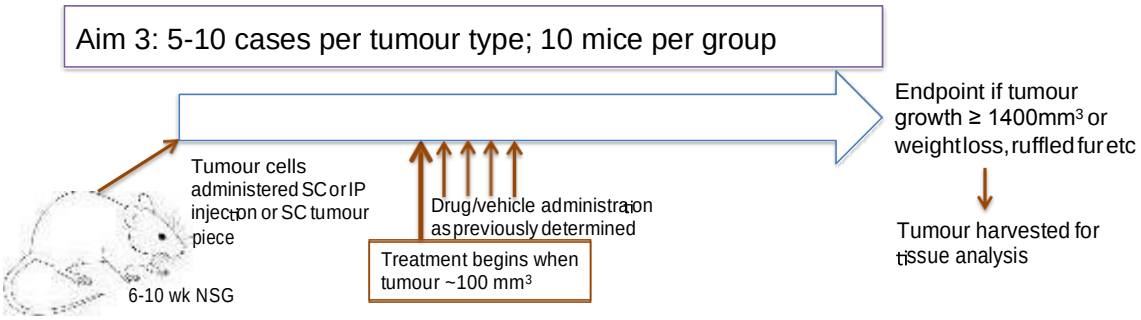
Drugs that have not been used at the Peter MacCallum Cancer Centre or not used in NSG mice will be tested in pilot studies to determine a tolerated dosing schedule. Briefly, we will treat non-implant/non- tumour bearing mice (n=3-5) with an expected non-toxic dose (one defined route and schedule). The mice will be observed closely throughout the day for any unexpected toxicity. On subsequent days the animals will be observed for signs of distress (e.g. signs of pain or distress such as lack of grooming, hunching and lethargy) and weighed daily (as the most common symptom of a toxic drug is weight loss) until the end of the treatment period and at least three times a week thereafter, depending on whether there is any weight loss. The dose will be increased in groups of 3-5 mice until up to 15% weight loss occurs. This is defined as the maximum tolerated drug dose. For subsequent studies in tumour-bearing animals we will use a drug dose that results in not more than 10% weight loss. The dosing schedule will vary depending on the compound and how it is expected to be handled by the animal (information is often provided by the supplier of the compound). If drugs are to be given in combination, the appropriate schedule for each compound will be used and pilot studies (n=3-5 mice) will be performed to test tolerability of the combination. Feedback on pilot studies of new drug and drug combinations will be provided to the AEEC for approval prior to commencing experiments in larger studies.

b) Drug administration. Therapies will be delivered via intravenous injection **(SOP 21.3.09)**, intraperitoneal injection **(SOP 21.3.65),** oral gavage **(SOP 21.3.13),** or in the drinking water at the doses and schedules to be determined depending on the drug selected. Drugs administered intravenously will be given no more than 3 times a week while drugs given by intraperitoneal injection or oral gavage may be given up to twice daily. In some circumstances it may be necessary to sequentially implant pumps to enable the duration of drug treatment to be

increased. In this case, the skin will be opened at the original site, the implanted pump gently removed using forceps, and a new pump implanted at the same site using the original procedure as described in **SOP 21.3.62.**

c) Drug studies on tumour bearing mice. Following passaging as above, tumours will be allowed to grow to ~100 mm^3 as measured by electronic calipers 2-3 times per week before being treated with the selected therapy or vehicle control (e.g. 0.9% saline, 0.5% methyl cellulose) for a defined period (e.g. 4-12 weeks). Ten mice per group will be used. Drug will be administered with the known or previously established (as described above) route and dosing schedule.

Mice will be monitored 2-3 times per week as appropriate depending on the location/method of PDX (e.g. callipers for SC, weight/bloating for IP). Mice will be sacrificed either (i) when the total tumour volume (calculated as the (length x width2)/2) reaches ≥1400mm^3, (ii), if the skin over the tumour becomes necrotic or (iii) if the animals show signs of distress (eg. ruffled coat, lethargy, weight loss (≥20% starting weight), etc.) or (iv) if the volume of ascites is estimate to be >2mL (IP route). At end point, mice will be euthanized by CO_2 inhalation (**SOP 21.3.04**) or cervical dislocation (**SOP 21.3.06**). Tissue will be harvested from the mice for evaluation of drug effects (frozen and formalin-fixed).



GENERAL PROCEDURES TO BE USED

Housing and identification

Mice will be housed 5 per box. Where necessary mice will be ear-clipped (**SOP 21.3.19**) for identification and followed individually throughout the studies.

Surgical procedures

During all surgical/experimental procedures the surgical instruments, syringes, blades and suture needles will always be changed between each tumour sample/mouse to prevent cross-contamination or infectious spread. The surgeon and assistant will wear shoe covers, hair cover, clean gown, surgical mask and gloves. Nitrile gloves will be worn when handling and prepping the animals, and the surgeon will wear sterile surgical gloves when performing the surgery or handling anything in the sterile surgical field.

During surgical procedures and recovery phase mice will be kept on a heat pad to maintain good body temperature. For postsurgical pain management, 5mg/kg Rimadyl (Carprofen) will be injected subcutaneously on the hind flank, daily for 3 days post-surgery (**SOP: 21.3.46**)

Monitoring and experimental end points

Immediately following tumour inoculation mice will be monitored daily for three days and then up to 3 times weekly thereafter until the experimental end-point is reached (calculated as the (length x $width^2$)/2) of all tumours reaches ≥1400mm^3 or if the animals are showing sign of distress (e.g. ruffled coat, lethargy, weight loss (>20% starting weight), etc.) or other signs of disease (e.g. bloating). Mice will be euthanised by cervical dislocation (**SOP 21.3.06**), carbon dioxide (**SOP 21.3.04**).

2.3 Potential Benefit of the Project

Explain the significance and the potential benefit of the proposed project (maximum 100 words)
Advanced ovarian cancer, especially of the rarer non-high-grade serous subtypes, does not respond to conventional chemotherapy and outcome for patients is dismal. Very few patient-relevant *in vivo* models exist for these rare subtypes. We need to develop new models and use them to test new therapies. If we identify a therapy that is effective, including existing US Food and Drug Administration (FDA)-approved drugs, these could be offered to women with this disease and they may have improved overall survival.

2.4 Potential Impact on the Animals

2.4.1 List the procedures that will be performed on animals (bullet points) **Where possible please refer to SOPs** *(http://pmc-sps.petermac.org.au/sites/Research/policies/Animal%20Ethics/Forms/AllItems.aspx.*
• Ear-clipping for ID in mice (SOP 21.3.19) • Mouse Handling and Restraint (SOP 21.3.37) • Antibiotic Water Preparation and Use (Baytril 50) (SOP 21.3.77) • Euthanasia using cervical dislocation (SOP 21.3.06) • Euthanasia using carbon dioxide (SOP 21.3.04) • Euthanasia using anaesthetic overdose (SOP 21.3.80) • Anaesthetic- Isofluorane (SOP 21.3.54) • Monitoring Level of Anaesthesia (SOP 23.3.20) • Intraperitoneal injections (SOP 21.3.65) • Subcutaneous injections (SOP 21.3.66) • Intravenous injections (SOP 21.3.09) • Administration by gavage (SOP 21.3.13) • Tumour tissue xenograft enrichment model (SOP 21.3.73) • Carprofen (SOP 21.3.46) • Tumour growth and monitoring of mice including weighing SOP 21.3.36

2.4.2 Do experiments involve (please tick) *ANIMAL USE CATEGORIES*		
	~~**A**~~	**Observation of free-roaming animals only** *(not applicable at this facility)*
☒	**B**	**Immediate euthanasia of animals to obtain tissue for biochemical analysis, or *in vitro*, cell, tissue or organ studies.**
☐	**C**	**Experiments under anaesthesia, without recovery (i.e. animals are fully anaesthetised for the duration of the experiment, and are killed at its conclusion without recovery from anaesthesia)**
☒	**D**	**No anaesthesia, minor procedures used (e.g. injections, blood sampling, antibody raising, minor dietary manipulations)**
☐	**E**	**Survival after an intervention which causes minor stress of short duration (eg. following biopsies or cannulations).**
☒	**F**	**Survival after an intervention which causes major or prolonged stress (eg. major surgery, exposure to heat, cold, ionizing radiation; administration of toxic drugs; genetic manipulation, neoplasia or fetal intervention.**

☐	G	Other (please describe the impact on animals below)

2.4.3 Please indicate if the project involves any of the following:	
No	Death as an end point (as defined in the Code) * the AEEC approved project will need to be forwarded to Bureau of Animal Welfare under Regulation 12 (2) for final approval.
No	Creation of hybridoma
No	Production of monoclonal antibodies by ascites method
No	Prolonged restraint or confinement

2.4.4 Have any of the animals been the subject of a previous research or teaching activity?		
☒	No	
☐	Yes	If yes, provide AEEC Register Number/s of the other project/s, describe what was done to the animals previously and justify their use in this project.
	<insert text here>	

2.5 Replacements, Reductions and Refinements

The Code specifies that techniques that totally or partially replace the use of animals for scientific purposes must be sought and used wherever possible. Suitable websites and databases that could be useful are:

http://altweb.jhsph.edu/

http://www.nc3rs.org.uk/

http://oslovet.norecopa.no/dokument.aspx?dokument=80&mnu=about_us

2.5.1 Justify your choice of animal (species/strain/sex/age).

The mouse is a widely accepted model for pre-clinical studies of ovarian cancer therapies.

We need a mouse strain lacking a functional immune system to prevent xenograft rejection. NSG mice are the most commonly used strain for this type of experiment. We elect to use female mice since this is an ovarian cancer disease and the presence of gynaecological anatomy is important. Tumour inoculation will be performed in young adult mice (approx. 6-10 wks) as they will need to be aged for 10 months

2.5.2 Have alternatives that totally or partially replace the use of animals been incorporated into this project?		
☐	No	If no, provide a list of potential alternatives and explain why they are unsuitable for use in this project.
☒	Yes	If yes, please describe what alternatives are to be used in this project.
	All therapies to be evaluated will first be tested using *in vitro* models. Only therapies showing efficacy *in vitro* will be tested using the mouse models. Thereafter, there is no	

viable alternative to using animals as the primary aim is to understand what occurs *in vivo* (i.e. in the intact animal) and such experiments cannot be done in humans. Where possible, we will use common control mice for multiple experiments (e.g. using one group of controls to compare the effect of multiple treatments in Aim 3). Further, where applicable, we will perform pilot experiments on small numbers of mice before proceeding to larger cohorts.

2.5.3 Is this proposal a repeat of an earlier project?

☒	**No**	
☐	**Yes**	If yes, please explain why repetition is necessary.
	<insert text here>	

2.5.4 Please justify the number of animals requested in terms of statistical considerations and/or other considerations in the experimental design.

Please present this information in a table detailing number of treatment groups/number of mouse strains / conditions etc.

Aim 1 Pilot – first 5 cases		
NSG	IP injection	3-6 mice per case (n=5) per tumour type* (n=4), maximum of 5x6x4 = **120**
NSG	SC/Matrigel	3-6 mice per case (n=5) per tumour type (n=4), maximum of 5x6x4 = **120**
NSG	SC/implantation	3-6 mice per case (n=5) per tumour type (n=4), maximum of 5x6x4 = **120**
NSG	IM implantation	3-6 mice per case (n=5) per tumour type (n=4), maximum of 5x6x4 = **120**
NSG	Testing cell lines/ tumour organoids (may not be required if above is successful)	3-6 mice per case (n=5) per tumour type (n=4), maximum of 5x6x4 = **120**
Aim 2 – next 5 cases		
NSG	Best method from first 5	3-6 mice per case (n=5) per tumour type (n=4), maximum of 5x6x4 = **120**
Serial transplantation		
NSG	Passage maximum of 2 tumours from each case (n=10 per tumour type) into 6 mice each	At most 10 cases per tumour type. Maximum of 6x10x4 = **240**

Aim 3 Drug treatment		
NSG	Drug tolerability up to 8 drugs/combinations	Up to 8 drugs x 5 mice =**40**
NSG	**10** mice per group, **three** groups per PDX (2 treatment, one control) and **four** experiments (total 8 drugs tested per case). Assume **6** PDX models over 3 years to be assessed for therapy. Expect this will only be for **two** tumour types (MOC/LGSC) in this time frame.	10 x3 x4 x6 x2 = **1440**
		2440

**** The four tumour types refer to ovarian cancer subtypes: mucinous, low-grade serous, endometrioid and clear cell.***

Please indicate whether a statistician / biometrician was consulted about the design of this project.

We have based our research plan on previous experience by others and have not specifically consulted a statistician/biometrician about this particular submission.

However, we used the Boston University Institutional Animal Care and Use Committee (IACUC) spreadsheet to estimate power. Given a 30% difference in tumour size (e.g. 1400 vs 1000 +/- standard deviation of 20%) 10 mice per group gives us 95% power to detect this difference at alpha = 0.05.

2.5.5 To reduce animal use, would the animals or their tissues, at the conclusion of your experiments, be suitable for use in another project? If not, please explain why not.

No. All mice will have been inoculated with tumour cells and would not be suitable for other experiments.

2.5.6 Does the project involve the use or production of genetically modified animals e.g. transgenic, knockout or of animals with spontaneous genetic mutations?

☐ **No**

☒ **Yes**

- ☐ No
- ☐ Yes,,OGTR PC1 Notifiable Low Risk Dealing (eg knockout and transgenic mice if new mice to Peter Mac)
- ☒ Yes, Naturally occurring mutant (eg SCID, nude)
- ☐ Yes, Other (specify PC2 Notifiable Low Risk Dealing (NLRD) or Dealing Not Involving Intentional Release (DNIR))

  Please check with the Chair or Secretary of the Institutional Biosafety Committee if unsure whether PC2 NLRD or DNIR coverage is needed

Strain	Phenotype report details
NSG	*Previously submitted.*

2.6 Location, Housing and Monitoring of Animals

Investigators are responsible for monitoring the welfare of their animals. This responsibility begins when an animal is allocated to the approved project and ends with the specified fate of the animal at the completion of the project.

Unexpected incidents that impact on the welfare of any individual animal or group of animals must be responded to immediately and reported to the AEEC.

All personnel identified in this section of the proposal must be aware of the criteria for monitor welfare of the animals and of how records are to be kept.

For housed animals, welfare monitoring checklists must be kept with the animals so as to be readily accessible to all nominated personnel, animal facility staff and the AEEC if requested.

2.6.1 What type of housing will be used? Describe any special housing requirements.

Mice will be housed in the Peter MacCallum Cancer Centre animal facilities on Level 8 in the Victorian Comprehensive Cancer Centre, Parkville (Standard IVC caging).

2.6.2 Will any animals need to be housed individually?

☒	**No**	
☐	**Yes**	If yes, explain why, how long and how the impact of social isolation be minimised?
	<insert text here>	

2.6.3 Where will the procedures be performed? If mice need to be transported from where they are housed to where the procedures are carried out please provide details of how this will be done.

All procedures will be carried out in the rooms within the animal facilities on Level 8.

2.6.4 Day-to-day monitoring: Who will monitor the animals during the project?

Weekdays:

The individual researcher associated with that experiment (indicated on the cage card) will be responsible for day-to-day monitoring. If they are not available, Dr Jessica Beach or Dr Liz Christie should be contacted.

After hours (including weekends and holidays):

Animals will be monitored after hours firstly by rostered animal house staff. Should an issue with a mouse arise, the individual researcher associated with that experiment (indicated on the cage card) should be contacted. If they are not available, Dr Jessica Beach or Dr Liz Christie should be contacted.

2.6.5 Have you provided a weekend/after hours protocol for animal facility staff?		
☒	**Yes**	Please attach this protocol to this application

2.6.6 Have you attached monitoring checklists?	
☒	**Yes**

2.6.7 What clinical, behavioural or other signs will be used to indicate that intervention is needed to alleviate an animal's pain or suffering? What action will be taken if these indicators are reached?

(e.g. increase in the frequency of observations, administration of analgesics or other appropriate medication, withdrawal from the project, euthanasia etc?)

All experimental mice will be carefully monitored to minimise distress.

During surgical procedures we will pay close attention to the wellbeing of the animal. Prior to surgery we will examine the mouse for signs of insufficient anaesthesia (e.g. reaction to foot pad pressure by gently squeezing between fingers (SOP 21.3.20)). Surgery will only begin when the researcher is satisfied that a sufficient state of anaesthesia has been obtained (stage 3, e.g. decreased breathing rate, no response to stimuli). This state will be closely monitored during surgery and if necessary further anaesthesia will be applied if signs appear that anaesthesia is wearing off (e.g. movement, increased breathing rate). For pain management, 5mg/kg Rimadyl (Carprofen) will be injected subcutaneously on the hind flank, daily for 3 days post-surgery (**SOP: 21.3.46**).

Immediately post-surgery, mice will be housed in the warming cabinet until they have recovered from anaesthesia. Researchers will monitor the mice during the recovery period until they are conscious and mobile. In the rare event (<1 out of every 200 mice, <0.5% based on others past experience) that a mouse does not recover from anaesthesia (e.g. no signs of breathing, "bluish" skin, cold to touch) they will be removed from the container, have their neck dislocated and disposed of in yellow biological hazard waste bins. Any such occurrence will be recorded as an adverse event. Researchers will continue to monitor the mice on the two days following surgery. In particular we will look for inflammation, bleeding or infection around the surgical site, signs of distress and abnormal behaviour such as hunching, lack of grooming, lethargy or hyperactivity, and abnormal gait. Mice will already be receiving analgesia in this period as described above, but if signs of distress are evident interventions such as providing Ensure/nectar or placing animals on a heat mat will be employed, and the animal carefully monitored. Researchers will also liaise with the experienced animal house staff to determine an appropriate course of action. If these interventions do not improve the condition of the animal within 24 hours or if signs of distress worsen the animals will be euthanised.

After the initial recovery period researchers will check the mice every other day, at which time the mice will be weighed to monitor weight gain/loss. This will be in addition to the routine monitoring by animal house staff. If total tumour volume (calculated as the (length x width^2)/2) of all tumours reaches ≥1400mm^3 the animals will be euthanized. If other signs of disease such as weight loss (>20% starting weight)) or bloating are observed, mice will be euthanised.

At any point during the project, if mice show signs of distress: e.g. abnormal behaviour such as hunching, lack of grooming, ruffled fur, lethargy or hyperactivity, and abnormal gait, interventions such as providing Ensure/nectar or placing animals on a heat mat will first be employed, and the animal carefully monitored. Subcutaneous administration of Carprofen will be used immediately (SOP 21.3.46). Researchers will also liaise with the experienced animal house staff to determine any other appropriate course of action. If these interventions do not improve the condition of the animal within 24 hours or if signs of distress worsen the animals will be euthanised.

2.7 Fate of the Animals

2.7.1 What will happen to the animals at the completion of this project?
It is expected that all animals will be euthanised at the end of each experiment for tissue collection.

2.7.2 If animals are to be killed, how will this be done? *Where appropriate, please refer to an SOP.*
Mice will be euthanised by cervical dislocation **(SOP 21.3.06),** carbon dioxide **(SOP 21.3.04)**.

2.7.3 What will be the method of disposal of dead animals?
Mice will be stored at -20ºC prior to being incinerated.

SECTION 3 PERSONNEL INFORMATION

Investigators have personal responsibility for the welfare of the animals they use and must act in accordance with all requirements of the Act, the Regulations, the Code and the AEEC. This responsibility begins when an animal is allocated to the approved project and ends with the specified fate of the animal at the completion of the project.

3.1 Principal Investigator

Name *(title, given name, family name)*	Dr Kylie Gorringe		
Position	Team Leader		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☐	If yes, complete details below.
	No	☒	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
			☐ **Yes** ☐ **No**
			☐ **Yes** ☐ **No**
			☐ **Yes** ☐ **No**
			☐ **Yes** ☐ **No**
			☐ **Yes** ☐ **No**
			☐ **Yes** ☐ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

3.2 Other Investigators

Name *(title, given name, family name)*	Ms Carolina Salazar		
Position	PhD Candidate		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures (**Individual techniques and procedures must be listed**)	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** *NB: where procedures have been performed more than 20 times indicate '>20'*	**Training required?**
SOP 21.3.37 Mouse Handling and Restraint[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.06 Euthanasia using cervical dislocation[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.04 Euthanasia using carbon dioxide[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.54 Anaesthetic- Isofluorane[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.65 Intraperitoneal injections[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.66 Subcutaneous injections[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.09 Intravenous injections[2]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.13 Administration by gavage[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.73 Tumour tissue xenograft enrichment model[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.46 Carprofen[1]	**0**	**0**	☒ **Yes** ☐ **No**
Tumour growth and monitoring of mice including weighing[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.36 Daily Observation[1]	**0**	**0**	☒ **Yes** ☐ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Animal Facility Induction will be undertaken first, followed by General Animal Handling Training by Animal Facility staff until they are satisfied with her competency.
Dr Jessica Beach will be training Carolina for most techniques (indicated by #1 above), for which she has >3-5 years' experience.
#2 Intravenous injections will be taught by Michael Durrant (> 3 years' experience).
IM implantation of tumour (part of 21.3.73) will be taught by Dr Nick Clemons or one of his experienced lab members or animal facility staff member, who will have performed the technique > 20 times.

Name *(title, given name, family name)*	Dr Dane Cheasley		
Position	Post-doctoral researcher		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.37 Mouse Handling and Restraint	**7**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.06 Euthanasia using cervical dislocation	**7**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.04 Euthanasia using carbon dioxide	**7**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.54 Anaesthetic- Isofluorane[1]	**0.5**	**5**	☒ **Yes** ☐ **No**
SOP 21.3.65 Intraperitoneal injections[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.66 Subcutaneous injections[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.09 Intravenous injections[2]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.13 Administration by gavage[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.73 Tumour tissue xenograft enrichment model[1]	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.46 Carprofen[1]	**0**	**0**	☒ **Yes** ☐ **No**
Tumour growth and monitoring of mice including weighing	**7**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.36 Daily Observation	**7**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Animal Facility Induction will be undertaken first, followed by General Animal Handling competency assessment and additional training as required by Animal Facility staff until they are satisfied with his competency.

Dr Jessica Beach will be involved in training Dane for techniques where he lacks experience (indicated by #1 above), for which she has >3-5 years' experience.
#2 Intravenous injections will be taught by Michael Durrant (> 3 years' experience).
IM implantation of tumour (part of 21.3.73) will be taught by Dr Nick Clemons or one of his experienced lab members or animal facility staff member, who will have performed the technique > 20 times.

Name *(title, given name, family name)*	Dr Jessica Beach		
Position	Post-doctoral researcher		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.37 Mouse Handling and Restraint	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.06 Euthanasia using cervical dislocation	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.04 Euthanasia using carbon dioxide	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.54 Anaesthetic- Isofluorane	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.65 Intraperitoneal injections	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.66 Subcutaneous injections	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.13 Administration by gavage	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.73 Tumour tissue xenograft enrichment model	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.46 Carprofen	**>5**	**>20**	☐ **Yes** ☒ **No**
Tumour growth and monitoring of mice including weighing	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.36 Daily Observation	**>5**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Name *(title, given name, family name)*	Dr Liz Christie		
Position	Post-doctoral researcher		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.37 Mouse Handling and Restraint	**4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.06 Euthanasia using cervical dislocation	**0**	**0**	☒ **Yes** ☐ **No**
SOP 21.3.04 Euthanasia using carbon dioxide	**2**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.54 Anaesthetic- Isofluorane	**4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.65 Intraperitoneal injections	**4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.66 Subcutaneous injections	**4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.13 Administration by gavage	**1**	**5**	☐ **Yes** ☒ **No**
SOP 21.3.73 Tumour tissue xenograft enrichment model	**4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.46 Carprofen	**4**	**>20**	☐ **Yes** ☒ **No**
Tumour growth and monitoring of mice including weighing	**4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.36 Daily Observation	**4**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Dr Jessica Beach will train Dr Christie. She has >3-5 years' experience in most of the techniques required, and has performed all >20 times.

Name *(title, given name, family name)*	Dr Kara Britt		
Position	Group Leader (Junior Faculty)		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.37 Mouse Handling and Restraint	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.06 Euthanasia using cervical dislocation	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.04 Euthanasia using carbon dioxide	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.54 Anaesthetic- Isofluorane	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.66 Subcutaneous injections	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.13 Administration by gavage	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.46 Carprofen	**>10**	**>20**	☐ **Yes** ☒ **No**
Tumour growth and monitoring of mice including weighing	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.36 Daily Observation	**>10**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Fully trained for all procedures listed above

Name *(title, given name, family name)*	Dr Nicholas Clemons		
Position	Group Leader (Junior Faculty)		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.37 Mouse Handling and Restraint	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.06 Euthanasia using cervical dislocation	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.04 Euthanasia using carbon dioxide	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.54 Anaesthetic- Isofluorane	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.65 Intraperitoneal injections	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.66 Subcutaneous injections	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.13 Administration by gavage	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.73 Tumour tissue xenograft enrichment model	**>5**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.46 Carprofen	**>10**	**>20**	☐ **Yes** ☒ **No**
Tumour growth and monitoring of mice including weighing	**>10**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.36 Daily Observation	**>10**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Fully trained for all procedures listed above

Name *(title, given name, family name)*	Stephanie Le		
Position	Senior Animal Technician		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.06 & SOP 21.3.04 Euthanasia of adult mice by cervical dislocation or carbon dioxide	>3	>20	☐ **Yes** ☒ **No**
SOP 21.3.64 Intravenous injection of reagents and cells into mice	>3	>20	☐ **Yes** ☒ **No**
SOP 21.3.19 Ear/tail clipping of mice	>3	>20	☐ **Yes** ☒ **No**
SOP 21.3.77 Antibiotic Water Preparation and Use	>3	>20	☐ **Yes** ☒ **No**
SOP 21.3.13 Oral gavage	>1	>20	☐ **Yes** ☒ **No**
SOP 21.3.36 Tumour growth and monitoring in mice	>3	>20	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Fully trained for all procedures listed above

Name *(title, given name, family name)*	Lauren Mathews		
Position	Senior Animal Technician		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.06 & SOP 21.3.04 Euthanasia of adult mice by cervical dislocation or carbon dioxide	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.64 Intravenous injection of reagents and cells into mice	**>4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.19 Ear/tail clipping of mice	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.77 Antibiotic Water Preparation and Use	**>4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.13 Oral gavage	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.36 Tumour growth and monitoring in mice	**>3**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Fully trained for all procedures listed above

Name *(title, given name, family name)*	Shellee Brown		
Position	Senior Animal Technician		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures (**Individual techniques and procedures must be listed**)	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** *NB: where procedures have been performed more than 20 times indicate '>20'*	**Training required?**
SOP 21.3.64 Intravenous injection of reagents and cells into mice	**>4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.19 Ear/tail clipping of mice	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.77 Antibiotic Water Preparation and Use	**>4**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.13 Oral gavage	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.6 Tumour growth and monitoring in mice	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.06 & SOP 21.3.04 Euthanasia of adult mice by cervical dislocation or carbon dioxide	**>3**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Fully trained for all procedures listed above

Name *(title, given name, family name)*	Michael Durrant		
Position	Senior Animal Technician		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures **(Individual techniques and procedures must be listed)**	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** ***NB: where procedures have been performed more than 20 times indicate '>20'***	**Training required?**
SOP 21.3.06 & SOP 21.3.04 Euthanasia of adult mice by cervical dislocation or carbon dioxide	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.64 Intravenous injection of reagents and cells into mice	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.09 Intravenous injection	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.13 Oral gavage	**>3**	**>20**	☐ **Yes** ☒ **No**
SOP 21.3.36 Tumour growth and monitoring in mice	**>3**	**>20**	☐ **Yes** ☒ **No**

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (# years / # times procedure has been performed).

Fully trained for all procedures listed above

SECTION 4 PRINCIPAL INVESTIGATOR'S DECLARATION

I hereby declare that:

I have read Part IV of the *Prevention of Cruelty to Animals Act 1986* (the Act). the Regulations (as amended) and the current version of the *Australian code of practice for the care and use of animals for scientific purposes* (the Code). and accept the responsibilities detailed therein.

I understand that scientific activities involving the use of animals must not start before written approval from the AEEC is received.

I accept responsibility for the conduct of all experimental procedures detailed in this application. in accordance with requirements of the Act. Regulations (as amended). the Code. the Animal Ethics Committee.

I have listed each person engaged in this project under Section 3 and consider that they have the qualifications. experience and training appropriate for their role in the project: and that they are competent to perform procedures described to the extent of their role. If any person is not already skilled in the procedures. I will ensure that they obtain all necessary training in advance of performing any procedure independently. All personnel have been made aware of their role and responsibilities in this project. and have been given copies of all necessary documentation.

The Animal Facility Manager has been made aware of requirements for this application.

Principal Investigator's Signature:	
Date:	7/11/18

SECTION 5 OTHER INVESTIGATOR'S DECLARATION

I hereby declare that:
I am familiar with Part Ill of the Prevention of Cruelty to Animals Act 1986 (the Act). associated Regulations (as amend\;!d) and the current version of the Australian code of practice for the care and use of animals for scientific purposes (the Code) and accept the responsibilities detailed therein to extent of my involvement in this project.
I accept responsibility for the conduct of all experimental procedures detailed in this application that I undertake. in accordance with the requirements of the Act. the Regulations and the Code and the Animal Ethics Committee.

Investigator Name	Investigator Signature	Date
Carolina Salazar		
Dane Cheasley		8/11/18
Stephanie Le		
Shellee Brown		3.12.18
Michael Durrant		3/12/18
Dr Kara Britt		7/11/18
Dr Nicholas Clemons		8/11/18
Lauren Matthews		3/12/18
Dr Jessica Beach		
Dr Liz Christie		7/11/18

Tumour measurements (mm^2) & Observations/appearance of mice

Name …….. Lab …Gorringe……. Ethics # .....

Experiment # and title ……

Groups	Mouse #	Date				Date				Date			
		Tumor size (for s.c. or i.m tumours)	Code	Weight	Ascites >2ml Y/N	Tumor (for s.c. or i.m tumours)	Code	Weight	Ascites >2ml Y/N	Tumor size (for s.c. or i.m tumours)	Code	Weight	Ascites >2ml Y/N

Codes: X (culled), C (cold to touch), D (distress), E (end of experiment), H (Hunched), I (Infection), A (Inactive), L (Limping), OK (mouse is healthy and OK), O (Obese), R (ruffled fur), SB (slow/laboured breathing), S (skin pale), (tissues taken for experiment), TR (Tremors), W (weight loss), V (vocalization abnormal). Lesions/ulcers not weeping: sm (small), med (medium), la (large); U (ulcerated tumour, weeping). Kylie Gorringe will be contacted immediately if any of the above symptoms are encountered (except OK) and Kylie will decide whether mice should be subjected to increased monitoring, typically twice daily.

Humane endpoints: Mice will be euthanized if showing any of the following endpoints: severe diarrhoea, severe hunching, profoundly lethargic/inactivity, grossly obese, severely ruffled fur, continued tremors, ulcerated weeping tumours, tumour size ≥1400 mm^3. 2 ml of ascites will be estimated by an increase in body weight of >2 grams in excess of that anticipated from normal weight gain achieved in aging, as evident from age-matched control mice.

MONITORING SHEET – POST SURGICAL

AEEC NUMBER:
Principal Investigator: Kylie Gorringe **Phone: 85596521** **After Hours Phone: 0425729246**

Animal I.D.#	SURGICAL SITE APPEARANCE							BODY CONDITION							BEHAVIOUR						
Date																					
Time																					
Weight (g)																					
Surgical Site Appearance																					
Body condition																					
Behavior																					
Signature																					
Animal I.D.#																					
Date																					
Time																					
Weight (g)																					
Surgical Site Appearance																					
Body condition																					
Behavior																					
Signature																					
Animal I.D.#																					
Date																					
Time																					
Weight (g)																					
Surgical Site Appearance																					
Body condition																					
Behavior																					
Signature																					

SIGNIFICANT INDICATORS	**0**	Normal – No swelling, no redness	**0**	Normal	**0**	Normal
	1	Mild redness, no swelling	**1**	<10% weight loss	**1**	Minor changes
	2	Mild swelling of wound	**2**	10-15% weight loss	**2**	Abnormal, reduced mobility
	3	Redness and swelling of wound	**3**	>20% weight loss	**3**	Moribund
	4	Loss of wound clip and/or separation of wound edges				

Intervention Points *(These are the clinical sign, or combination of clinical signs, that will indicate that a specific action must be taken)*	**Action** *(e.g. increased observation frequency, treatment, withdrawal from the study)*
When a total score of two or more (in any single category) is reached, based on above checklist.	Provide Ensure/nectar; place animal on a heat mat. Increase observation frequency to twice daily. Notify Jessica Beach or Liz Christie
Humane Endpoints *(The clinical sign(s), indicating that the animal will be euthanized)*	**Method of euthanasia**
Any of tumour size ≥1400mm^3; >20% weight loss; severe bloating associated with increase in body weight >2g. If previously reaching intervention point,	Cervical dislocation (**SOP 21.3.06**) or carbon dioxide (**SOP 21.3.04**).

animals with worsening condition.	

Weekend/ After Hours Protocols For Sick/ Dead/ Distressed Mice

Date:
Ethics Project Number:

Contact 1
Name: Jessica Beach
Group/Lab Head: David Bowtell
Contact: Jessica Beach **Contact Number/s** @ **Peter Mac:** ext. 96503 (office)
Mobile/Pager: 0412 165 699

Contact 2
Name: Liz Christie
Group/Lab Head: David Bowtell
Contact: Liz Christie **Contact Number/s** @ **Peter Mac:** ext. 96505 (office)
Mobile/Pager: 0409 239161

Contact 3
Name: Kara Britt
Group/Lab Head: Ian Campbell
Contact: Kara Britt **Contact Number/s** @ **Peter Mac:** ext. 97110 (office)
Mobile/Pager: 0431 060258

Contact 4
Name: Dane Cheasley
Group/Lab Head: Ian Campbell
Contact: Dane Cheasley
Contact Number/s @ **Peter Mac:** ext. 64065 (lab)
Mobile: 0408 977 421

PLAN OF ACTION IF CONTACT/ S NOT REACHED:

Sick Mice:
If contacts are unreachable, kill by cervical dislocation and place in a clearly labelled dead bag in the fridge. Send email to inform researcher that the mouse needed to be euthanised (Jessica.beach@petermac.org, liz.christie@petermac.org, kara.britt@petermac.org, dane.cheasley@petermac.org and kylie.gorringe@petermac.org).

Dead Mice:

Dead mice are to be placed in a labelled dead bag and stored in the fridge. Send email to inform researcher that the mouse was found dead (Jessica.beach@petermac.org, liz.christie@petermac.org, kara.britt@petermac.org, dane.cheasley@petermac.org and kylie.gorringe@petermac.org).

OFFICE USE ONLY

Minor amendments may be approved by the Executive Sub-committee of the AEEC. All minor amendments approved by the Executive on behalf of the AEEC must be ratified by the full committee at the next meeting.

AEEC Project Number: E618
Principal Investigator: Dr Kylie Gorringe
Amendment Date: 17Jun2019
Amendment Title: Additional Co-investigator (Dall)

1 APPROVED BY THE CHAIR OF THE AEEC

28/06/19

Professor Phillip Darcy, Chairman **(Date)**
AEEC, PMCC

2 APPROVED AND/OR RATIFIED BY THE AEEC ON THE____/__/___

_____ - / /

Professor Phillip Darcy, Chairman **(Date)**
AEEC, PMCC

APPLICATION FOR ADDITIONAL CO-INVESTIGATOR
ANIMAL EXPERIMENTATION ETHICS COMMITTEE

Amendment Submission Date	17th June 2019
AEEC Project Number	E618
AEEC Project Title	New patient-derived mouse models for ovarian cancer
AEEC Approval Permit Dates	01/11/18 to 31/10/21
Principal Investigator	Kylie Gorringe

1 DETAILS OF ADDITIONAL CO-INVESTIGATOR

Name *(title, given name, family name)*	Dr Genevieve Dall		
Position	Postdoctoral researcher		
Will the investigator be carrying out techniques/procedures on live animals?	**Yes**	☒	If yes, complete details below.
	No	☐	If no, details of expertise are not required.

Proposed techniques / procedures (**Individual techniques and procedures must be listed**)	**Number of years' experience doing the procedure**	**Total number of times the procedure has been performed** *NB: where procedures have been performed more than 20 times indicate '>20'*	**Training required?**
SOP 21.3.37 Mouse Handling and Restraint	8	>20	☐ Yes ☒ No
SOP 21.3.06 Euthanasia using cervical dislocation[1]	0	0	☒ Yes ☐ No
SOP 21.3.04 Euthanasia using carbon dioxide	8	>20	☐ Yes ☒ No
SOP 21.3.54 Anaesthetic- Isofluorane	7	>20	☐ Yes ☒ No
SOP 21.3.65 Intraperitoneal injections	6	>20	☐ Yes ☒ No
SOP 21.3.66 Subcutaneous injections	6	>20	☐ Yes ☒ No
SOP 21.3.09 Intravenous injections[1]	0	0	☒ Yes ☐ No
SOP 21.3.13 Administration by gavage	5	>20	☐ Yes ☒ No
SOP 21.3.73 Tumour tissue xenograft enrichment model[2]	0	0	☒ Yes ☐ No

SOP 21.3.46 Carprofen[2]	0	0	☒ Yes ☐ No
Tumour growth and monitoring of mice including weighing	6	>20	☐ Yes ☒ No
SOP 21.3.36 Daily Observation	8	>20	☐ Yes ☒ No

2 DETAILS OF TRAINING

Please provide details of who will be training the investigator on the proposed techniques/procedures. Please specify their experience (include number of years experience).

Intravenous injections and cervical dislocation will be taught by Michael Durrant – indicated by #1 above. (> 3 years' experience).

Dr Liz Christie will be involved in training Gen for techniques where she lacks experience (indicated by #2 above), for which she has >3-5 years' experience. IM implantation of tumour (part of SOP 21.3.73) will be taught by Dr Nick Clemons or one of his experienced lab members or animal facility staff member, who will have performed the technique >20 times.

- *PLEASE NOTE; THE AEEC MUST APPROVE THE ADDITION OF THIS NEW CO-INVESTIGATOR TO THE PROJECT BEFORE HE/SHE MAY COMMENCE WORK OR TRAINING INVOLVING ANIMALS.*

DECLARATION OF CO-INVESTIGATOR:

We have read the current NHMRC "Code of Practice for the Care and Use of Animals in Research in Australia', Part 3 of the 'The Victorian Prevention of Cruelty to Animals Act 1986', Regulations (as amended), and the original protocol. We accept responsibility for the conduct of the experimental procedures detailed in this application in accordance with the above documents and any other conditions imposed by the AEEC.

14/06/19

…… ……..
Signature Co-Investigator Date

DECLARATION OF PRINCIPAL INVESTIGATOR

I certify that all personnel involved in this project are appropriately qualified and experienced, or will undergo the appropriate training, to perform the procedures required of them. I have provided the investigator with a copy of the original application and amendments.

Kylie Gorringe  17-6-19

…… ……..... ……..
Print Name Signature Date