23 October 2020

Tara Jackson

By email: fyi-request-13728-609325e6@requests.fyi.org.nz

Dear Tara

I am writing in response to your information request dated 9 September 2020 under the Official Information Act (Act).

You have asked for a copy of the approved application(s) made to our Animal Ethics Committee on the use of the animals who were imported into New Zealand by University of Otago to be used for RTT purposes in 2018.

Decision

We have decided to grant your request.

We attach a copy of the approved application within scope of your information request.

Having given careful attention to public interest considerations in accordance with section 9(1) of the Act, we have redacted some parts of the attached application where we consider that good reasons exist for withholding information. We consider that these redactions are necessary to:

- protect employees of the University (including employees of organisations working alongside the University in relation to the approved application) from improper pressure or harassment, consistent with section 9(2)(g)(ii) of the Act. These redactions include the names and other identifying details of the principal investigators and researchers; and

- enable the University to carry out, without prejudice or disadvantage, commercial activities, consistent with section 9(2)(i) of the Act, and also to protect information where the making available of the information would be likely unreasonably to prejudice the commercial position of the person who supplied or who is the subject of the information, consistent with section 9(2)(b)(ii) of the Act. The approved application contains confidential information which is commercially sensitive, and this information is still being used for ongoing research purposes.

If you are not satisfied with our response to your information request, section 28(3) of the Act provides you with the right to ask an Ombudsman to investigate and review this response. However, we would welcome the opportunity to discuss any concerns with you first.

Yours sincerely

M Mendis

Mayhaka Mendis
Manager, Policy and Compliance
Office of the Registrar

Animal Use Protocol

1. General Information

AUP: AUP-18-124
Title: Investigation of the effectiveness of [redacted] in a mouse model of breast cancer

1.2 General Information

1.2.1 Description of animal use

Check the one box that best describes this protocol.

Research

1.2.2 Protocol involves

Check the boxes below which are relevant to this protocol – multiple (or no) boxes may be checked.

The use of physical or chemical hazards
The administration of substances to animals
Survival surgery
Multiple survival surgeries for any animal

1.2.3 Lay description of research/teaching plan and the ethical cost/benefit involved such that the reviewer gains an understanding and overiew of the whole project.

Please provide a brief summary (150 words) in LAYPERSON's terms for each question.

A) Detail the overall purpose and significance of this project.
Answer

Oestrogen receptor positive breast cancer accounts for over three quarters of the approximately 3000 new breast cancer diagnoses in New Zealand each year. This proposal focuses improving outcomes for these patients by investigating whether the [redacted]

B) Specify how the well-being of the animals is likely to be affected
Answer

[redacted] to replicate this in animals we must ovarectomise the mice prior to commencing treatment. Mice will experience some discomfort following ovariectomies and this will be managed by appropriate pain medication. This model is susceptible to developing ulcers on the tumour, however our experience with this model means we are able to quickly identify ulcers and euthanise the mouse. We will carefully monitor the size of the tumour and the condition of the mice, and if at any time a mouse experiences adverse side effects, we will euthanise the mouse as soon as possible. This is a relatively short study and generally the well being of the mice is not adversely affected by the tumours.

The use of [redacted] are generally well tolerated in mice and our dose regimen has been chosen in consultation with other researchers who have used [redacted] to treat cancer in mice. [redacted]

The use of [redacted] is generally well tolerated in mice and our dose regimen has been chosen in consultation with other researchers who have used these [redacted] in mice, specifically [redacted]

C) Describe the potential benefits obtained from the use of animals in this project
Answer

The use of an animal model with an intact immune system is essential to study the immunomodulatory effects of [redacted] as these interactions cannot be fully recapitulated *in vitro*. An *in vivo* model will enable us to study the immunological response to tumour development and the way in which this is modified by therapy. This will enable us assess whether [redacted] may be a potential treatment for oestrogen receptor positive breast cancer.

[redacted] This may ultimately reduce the growth rate of the tumours. [redacted] are currently approved for use in humans so these findings could be readily translated into a human clinical trial and potentially provide a better standard of treatment for breast cancer patients.

[redacted] may ultimately reduce the growth rate of the tumours, providing insight into a potential therapy for this tumour type [redacted] are currently used in humans for other cancer types, so these findings could potentially lead to trials in breast cancer patients and provide a better standard of treatment. The interaction of [redacted] and this work will help assess how these treatments function together, lead to publications and provide further information we can use in other projects. [redacted]

D) Comment on any potential adverse attention that could arise as a result of activities undertaken during the proposed study which might compromise the reputation of the University of Otago and how this will be mitigated.

Please enter "N/A" if not applicable.

Answer

N/A

E) Has this application or one substantially like it been submitted to any other AEC (for example a Department of Conservation Committee or another University AEC) for approval?

This question is in regards to protocols submitted to any Animal Ethics Committee other than this one - it is not asking if this is a continuation of a previous approval. Please also advise if you have previously submitted to another University of Otago AEC.

No

F) Comment on how experimental findings will be used, promoted or published
Answer

We intend to publish a manuscript(s) describing the findings of the study in a peer reviewed journal, and present the work at a number of conferences including the New Zealand Society for Oncology Annual Meeting, the San Antonio Breast Cancer Symposium and the American Association for Cancer Research Annual Meeting. The results from this research will disseminated to local clinicians, media and breast cancer interest groups, as well as supporting applications for further funding and research.

G) Provide a description of the background and originality of this project for the Committee

List the specific aims and objectives of the animal experimentation. This should describe the experience of the animals in lay terms (NOT a cut and paste from a grant application). Any duplication of previous studies should be noted and fully justified. References should be included at the end of this section.

Answer

Breast cancer is the most common malignancy in women, accounting for more than 400,000 deaths per year worldwide. Oestrogen receptor positive breast cancer accounts for over three quarters of the approximately 3000 new breast cancer diagnoses in New Zealand each year. The majority of human breast carcinomas are classified as oestrogen receptor (ER) positive and are treated with therapies that stop the production or activity of the hormone oestrogen. Unfortunately, many patients receive little to no benefit from these treatments, [redacted]. This proposal focuses on improving outcomes for these patients by investigating whether the [redacted]. To date, investigation of these [redacted] has been limited. [redacted]. Our study will investigate further ways through which responses can be achieved.

Our specific aims are:

1) To determine whether [redacted]

2) To assess how these therapies affect the [redacted] of the tumours.

[redacted]

H) Has your research group or teaching team performed this type of experiment before?
Yes

I)Please indicate the source of grant support and whether already received or pending
Answer

[redacted]

1.3 Project Continuation

Is this a continuation of an ongoing or previous protocol?
Yes

In lay terms, explain the achievements of the previous project(s).

Provide AEC numbers, their significance and how the results have been disseminated (when citing references, please either attach document using the attachments button at the bottom of the page, insert a link, or include source i.e. journal title, year and page numbers).

Answer

[redacted]

2. Ethical Use of Animals

2.1 Rationale for Animal Use

Explain your rationale for animal use and make an argument why this study does not unnecessarily duplicate or replicate previous experiments done in this lab or by others.
Answer

The use of an animal model with an intact immune system is essential to study the immunomodulatory effects of these drugs as these interactions cannot be fully recapitulated *in vitro*. An *in vivo* model will allow us to use mice with an intact immune system, enabling the study of the immunological response to tumour development and the way in which this is modified by therapy. This will enable us assess whether treatment with [redacted] which has not been done before.

2.2 Reduction

Provide details on how you have reduced the number of animals you propose to use to the minimum compatible with achieving the purpose of the work?

This may have included basing numbers on a pilot experiment or prior research, or seeking expert statistical advice on group sizes.

Answer

Prior work demonstrates that the group sizes proposed will achieve significant results and the numbers chosen are based on explicit statistical advice. Each group will have 10 mice which has been powered to detect a standardised difference of 0.42 or greater in our immune cell markers ([redacted]) and [redacted] in the paired pre- and post treatment samples (90% statistical power). The tissues obtained from these experiments will be analysed by immunohistochemistry and a subset will be analysis using flow cytometry analysis. The primary endpoint of this phase of the study will be a difference in tumour area. Ten mice per group will give 90% power to detect a standardised difference of 0.23 or greater. We have added an additional two mice per group in the event some of the tumours ulcerate before the treatment is complete.

In addition, we are able to include results from some mice from a previous ethics [redacted]. There are four treatment groups which overlap with those in [redacted] and this application. In the interests of reducing the number of mice used, we have designed this application to allow us to directly compare results from treatment groups in this protocol with results from treatment groups in the [redacted]. These treatment groups include 1.Vehicle, 2. [redacted], 3 [redacted].

2.3 Refinement

Provide details on how you have sought to minimise the welfare impact of the manipulations you propose?

Consider refinements such as providing environmental enrichment; limiting studies to non-invasive behavioral studies; utilising non-invasive sampling or analytical techniques; minimising impact through the use of implants, less invasive surgical approaches and non-survival surgeries; the appropriate use of anaesthetics, and humane early endpoints.

Answer

The surgical approach we have chosen is designed to minimise tissue trauma and aseptic conditions are used to minimise infection as both will shorten post-operative recovery. Analgesics will be administered post-operatively twice daily (every ~12 hours) for 4 days to provide relief from pain or distress, and antibiotics will be administered pre and post-operatively for 1 day to minimise infection. The chosen early endpoint of tumours reaching the size of 200 mm^2 or tumour ulceration minimises or avoids suffering. Housing enrichment will be provided including plastic tubes, bedding nests, and chewing blocks which will enhance the environment and contribute to the welfare of the mice.

2.4 Replacement

Provide details on why non-sentient or non-living alternatives can not be used in this proposed study.

Discuss (where appropriate) why the following alternatives can not be used: computer, physical or mathematical models; microbial or cell culture; animals with less complex nervous systems.

Answer

Computer, chemical, physical or mathematical models cannot be appropriately applied to this model. Tissue culture cannot be appropriately applied because the immune system is a highly complex network that cannot be fully replicated *in vitro*. Furthermore we are interested in the interactions between the immune system and tumours which cannot be studied in *in vitro*. The processes studied cannot be effectively modelled using animals with a less complex nervous system and are effectively modelled by the chosen animal species.

3. Personnel

3.1 Principal investigator

[redacted]

3.2 Co-Investigators

[redacted]

3.3 Research personnel

[redacted]

3.4 Student Research Personnel

Not applicable

3.5 Facility Managers and Animal Care Personnel

Not applicable

3.6 Peer-Reviewers

Not applicable

3.7 Signers

4. Species

4.1 Species

Mouse

MPI Impact Grade

MPI Impact Grade	Strain	Number of Animals
C - Moderate Impact	Mouse	72

Mouse

4.1 A) Species Justification/Information

Justify the appropriateness of the species selected for use in this protocol.

Why have you chosen to use this particular species as opposed to any other?

Answer

The processes studied are effectively modelled by the chosen species of animal. We need to use the 129SvEv mouse strain because [redacted] can only be transplanted into this strain or 129S6SvEv and there are currently no alternative [redacted] suitable for use as [redacted]. We are investigating the immune system in [redacted], therefore, we need our mouse model to be immunocompetent and syngeneic. The [redacted] were originally derived from the 129S6SvEv strain by [redacted].

[redacted]

[redacted] will import mice for us from [redacted], Australia. We have to import these from Australia because there are no New Zealand suppliers of either 129S6SvEv or 129SvEv mice . We requested to order 129S6SvEv male mice for breeding purposes, but the [redacted] would not allow this. Therefore, we are only allowed to purchase female stock mice for our studies. We are currently in the process of contacting alternative animal vendors such as [redacted] in the United States to try and order male 129S6SvEv mice so we can breed mice in Dunedin. The mice are not manipulated while in quarantine and all required approvals for the importation of these mice are in place and currently active.

In an attempt to find alternative models, our lab is currently in the process of characterising the [redacted] and can be grown in the immunocompetent syngeneic BALB/c mouse strain. These mice are more commonly used and easily obtainable so we can set up a breeding colony at University of Otago which will avoid the need to importation and quarantine. If successful, this might allow us to study [redacted] in an alternative model.

4.1 B) Animal Source

i) From where will animals be sourced?
Other
[redacted] will import mice for us from [redacted], Australia. We are importing stock females and the animals will not be manipulated while in quarantine. All required approvals for the importation of these mice are in place and currently active

ii) Have any of these animals been manipulated in a previous study?
No

4.1 C) Animal Characteristics

Describe the characteristics of the animals used in this protocol.

For example age, weight, size, gender, etc.

Answer

Mouse - 129S6/SvEv, Female, Adult, ~20 grams

SvEv
SvEv

5. Study Segments

5. Study Segments	4.1 Species & Strain
1. [redacted]	Mouse
2. [redacted]	Mouse
3. [redacted]	Mouse
4. [redacted]	Mouse
5. [redacted]	Mouse
6. [redacted]	Mouse

1. [redacted]

Species to be used for the study segment

Mouse

5.2.1 Study Segment Summary

Briefly explain the experimental design (is this a pilot or main study) and specify all animal manipulations used in this study segment.

*Specific manipulations to be employed in the study must be described on the "Manipulations" tab. The Study Segment Summary should be written in an **animal-centric** fashion to allow the AEC to understand the **experimental course of an animal from its entry into the experiment to the endpoint** of the study. A flowchart of the individual manipulations may be an effective presentation of the study segment. You can attach a flow chart using the attachments button at the bottom of the form.*

Answer

The following procedures will be used for the main study. To generate [redacted] adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, domitor and atropine) and a [redacted] mammary [redacted] will be injected subcutaneously into the mammary tissue. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring. Mice will be monitored at least twice weekly until tumours become palpable. Once palpable, tumours will be measured with calipers 2-3 times per week until the tumour reaches 49 mm^2. It usually takes about 20-40 days for the tumours to reach 49 mm^2

When a tumour reaches 49 mm^2, a 18-gauge core biopsy will be taken. Mice receive antibiotic (Amphoprim) prior to surgery and will be anaesthetised (Ketamine, Domitor, Atropine). An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive anaesthetic. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to staunch any bleeding and the skin sutured as required.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery, and mice will receive analgesics (Temgesic) twice daily for 3-4 days post-surgery. Mice in the [redacted] receive a [redacted] on the day of the operation and each day thereafter until day 25. After 3-4 days they will be replaced into a communal cage of 2-5 animals. Mice in the [redacted] will receive a [redacted] of sterile saline on the day of the operation and each day thereafter until day 25. Mice in the checkpoint [redacted] ([redacted]) will receive a [redacted] on days 3, 5, and 7 post-surgery for a total of 3 [redacted] per mouse. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out. At the end of 25 days, mice will be euthanised and a blood sample will be taken. We will collect the tumour, spleen and lymphoid tissues. The tissues obtained from these experiments will then be analysed to address the experimental objectives. Please see the attached flow chart illustration for further clarification

5.2.2 Study Segment

Is this study segment the same as any other study segment?
Yes

If the same manipulations are used in each segment, with the same species and the same adverse effects are anticipated, then this section only needs to be completed once.

Please advise which other study segments are the same as this one.

Segments 1-6

5.2.3 Adverse Effects

A) Please indicate if any of the following adverse effects may potentially occur during the proposed study.
Weight Loss
Haemorrhage
Infection
Pain
Other

Tumour ulceration

B) If any of the above adverse effects were selected then please specify how the adverse effect will be managed.

If you have not ticked any of the above adverse effects, please enter n/a

Answer

Adverse side effects, apart from ulceration of the tumour as discussed previously, are unlikely but if a mouse meets any of the standard international humane endpoints we will euthanize the animal immediately. Pain will be well managed with drugs following the biopsy of the tumour and ovariectomy. We have not experienced haemorrhage or wound infection following ovariectomy, but are a risk in all surgeries. Careful surgical technique and administration of antibiotics reduce this to a very low risk. Some weight loss may occur as the tumour increases in size and this will be monitored by regular weighing and assessment of the overall condition of the mouse, however this has not been noticed in any mice as of yet. With any application of anaesthesia to mice, there is likely to be some small amount of weight loss, as the activity and appetite of the mouse is reduced for several hours. However, we would expect less than 10% body weight loss, and the mice will be monitored with twice daily weight measurements for 4 days following surgery. Mice quickly recover the weight loss within a few days, and in the event weight loss is greater than the humane endpoints, the mouse will be euthanized.

C) Detail the survival rates and incidence of adverse events for the proposed manipulations based on the relevant literature and your experience of the manipulations
Answer

This [redacted] has been developed within the last 5 years and, to date, only appears in limited number of papers, which unfortunately do not investigate long term survival rates. Neither paper mention ulcers or adverse effects, however personal communication with one of the authors confirms that ulceration occurred in some cases. We have a successful tumour take rate of 94%, which is similar to other mammary xenografts.

Our efforts to refine the model including reducing the number of [redacted] have reduced the rate of ulceration amongst our mice to below 20% (down from ~25%). Some ulcers formed quickly before the tumour was large enough to begin treatment, and others developed after the initial biopsy at any time ranging from 4 - 23 days post biopsy and commencement of treatment. Ulcers may occur for a variety of reasons, and we have addressed each of these causes in the attempt to reduce ulceration. We have confirmed the [redacted] are free from bacteria and mycoplasma, and that the [redacted] into the mammary gland, not subcutaneously. We are using the smallest volume of injection possible under sterile conditions and prepare the tumour carefully for biopsy. We have explored the possibility of using alternative, more invasive, surgical techniques to implant tumours with the aid and supervision of the [redacted] in 2014 and in November 2017 but, to date these pilot animals showed that [redacted] into the mammary fat pad was more effective using our current method. We also discussed alternative approaches with [redacted] and [redacted] recommended this method was most effective, based on the large number of animals [redacted] had used mammary fat pad implantation on. We have also discussed a change to softer bedding with [redacted]. It is known that some cell lines are more prone to ulceration than others, and unfortunately it appears [redacted] are one of these, however there are no other suitable [redacted] that we can utilise for this study.

Ovariectomy is a routine procedure carried out in many mouse facilities around the world, and [redacted] has had very high survival rates following ovariectomy in our previous study. The tumour biopsy results in minor bleeding which is easily controlled and due to the small gauge of the needle there is only a small wound in the skin which heals well. Mice will be treated with antibiotics and painkillers and we expect minimal complications following surgery. Ovariectomy can cause adverse effects over a longer time period, however given the short length of the study, we do not expect any of these to occur.

[redacted] works by lowering the production of [redacted], and is unlikely to have any significant effects on the mice. Some papers have reported lower uterine weights and some changes in bone density, but as mice are only exposed to [redacted] for 25 days, we have not observed any significant effects on the mice. We have investigated the possibility of an osmotic micro pump to deliver [redacted]. However, the pumps available are only able to carry a sufficient volume of [redacted] to last 50 hours so would have to be refilled every second day which we believe negates the advantage of using the pump. In addition, the mice must be handled every day regardless of whether we use [redacted] or a pump. Currently, we are able to combine the weighing, monitoring, and injection into one event. Moreover, the concentration of [redacted] would need to be increased. Previously when using a higher concentration of [redacted], we observed a local skin reaction, which we were able to eliminate by reducing the concentration and injecting a larger volume. We are concerned that with a slow release of a small volume of concentrated drug in a fixed position, that there would be a local inflammatory reaction. The subcutaneous injections allow us to rotate the position of injection to different parts of the body to avoid repeatedly irritating the same site.

Our [redacted] will be prepared according the method developed by our collaborator at the [redacted] which has previously been shown to be well tolerated by mice. In our recent study [redacted] we have encountered some problems with lack of recovery after surgery which is probably due to the timing of the [redacted] and we have changed the timing of the [redacted] to prevent this from happening in the future.

5.2.4 Animal Monitoring

A) Please describe the frequency and duration of animal monitoring over the course of the study

Frequency = number of times per day, Duration = number of days

Answer
Prior to [redacted], mice will be checked a minimum of twice weekly. Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

B) Please attach an example of the monitoring sheet to be used. Please note all sheets (including electronic records) need to be available for review by the AWO upon request.
Other (Please attach a sample to this protocol by using the attachments button at the top of the form.)

C) What are the parameters to be monitored?
Body weight
Food/water intake
Level of activity
Diarrhoea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Body Condition
Coat rough, fur on end
Hunched Posture
Pain Behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**
- **Cheek bulge**

Other
Tumour size should not exceed 200 mm^2

5.2.5 Endpoints

A) Is death an endpoint?

Death as an endpoint, which in biomedical science terms means the animal is left to die as part of the experimental protocol without any alleviation of suffering or human intervention is generally ethically unacceptable and must be fully justified.

No – animals will be killed

B) Indicate any additional humane endpoint criteria (other than those listed above) that may apply to this study.
Please include any other information you can provide on euthanasia endpoints for these animals
Tumour ulceration

C) Experimental Endpoint: What will determine the experimental endpoint(s) of the study?

For example: state the specific time points, specific tumour size, etc.

Answer
Mice will be euthanized at the end of the 25 day drug treatment, or when the tumour reaches a maximum size of 200mm2, whichever comes first.

5.2.6 Euthanasia

A) Describe the method of killing and euthanasia that will be used

Note that some methods may require scientific justification

CO2 chamber
Cervical dislocation (please note that cervical dislocation is not acceptable for rodents over 200g body weight.)

B) Please select the method of ensuring death of the animal
Vital signs monitoring, cessation of heart beat and respiration

5.2.7 Breeding

Are you breeding animals with a potential adverse phenotype under this protocol?
No

5.2.8 Animal Identification Methods

Describe method and age at which animals are identified.
Answer

Each animal will be identified with a unique cage card number. In addition, each animal inside the cage will have an ear punch unique to that cage number which allows us to identify individual mice by a unique ID number assigned to that mouse by the research group. Each cage is allowed to house a total of 5 mice, thus, the ear punches will be as follows: 1.) No Mark (NM) 2.) Left Ear (LE), 3.) Right Ear (RE), 4.) Left Ear/Right Ear (LE/RE), and Left Ear x2 (LEx2). Our system allows for the least amount of ear punches possible while still maintaining uniqueness among 5 mice. This will help reduce pain to the mice by using less ear punches. For example, if only 3 mice are in a cage the ear marks chosen will always be 1.) NM, 2.) LE, and 3.) RE, resulting in a total of 2 ear punches total; as oppose to using 1.) LE, 2.) LE/RE, and 3.) LEx2, resulting in 5 ear punches total. We have previously tried using ear tags, but noticed that some of the mice developed a red, swollen, inflammed area at the site of the ear tag. Our study is investigating immunological responses in the animal, so the ear tags are not ideal for our studies as they may add in confounding factors regarding the immune response in the mouse. The animals will be identified at the time of subcutaneous [redacted].

5.2.9 Surgery

A) Will you be performing surgery on this study segment?
Yes

i) Will the surgery be survival or non-survival?
Survival surgery

Please describe the surgery to be performed

Please provide the following details on the surgery:

Details of the sterilisation of all equipment, implants, surgical drapes etc and of any re-sterilisation during surgery (between animals).

Details of PPE used and how it is sterilised.

Preparation of surgical site.

Full details of the surgery – including drugs used.

Details of type of ear bars used for stereotaxic surgery.

Details of implants (size and weight).

1st surgery: Tumour implantation

To generate [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, domitor and atropine) and a [redacted] injected percutaenously into the mammary tissue. The hair is removed by clippers and hair removal cream to assist in locating the nipple. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. The area is sterilized with 100% ethanol before the [redacted]. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring.

2nd surgery: Ovariectomy, biopsy and [redacted].

Mice will be anaesthetised for this procedure and at the same time, they will be ovariectomised, or receive a sham overiectomy (for those mice not receiving [redacted]), and have have their tumours biopsied. This is required to prevent any oestrogen production and ensure the success of the [redacted]. Mice receive analgesics (Temgesic) and antibiotics (Amphoprim) prior to surgery. An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive analgesic. Surgical instruments are autoclaved for each animal, or sterilised between animals using the Germinator 500 bead steriliser in room [redacted]. The drapes are single use, sterile, clear, plastic drapes sourced from the [redacted], and gloves are sterilised with 100% ethanol. Hair is removed from the skin with a combination of clippers and hair removal cream. The skin is prepped by washing the incision area from inside to out with betadine and then 100% ethanol. A clean gown, hair net and facemask are worn. The biopsy guns are surgical grade and are also sterilized with 100% ethanol between uses. The biopsy guns are typically reused 5 times before being discarded. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. A small incision is made in the abdominal wall and the ovary and oviduct removed before closure with sterile sutures. The abdominal wall and skin are closed separately. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to stauch any bleeding and the skin sutured as required. On the following day, treatment with [redacted] or vehicle will be commenced. [redacted] (or a vehicle control) will be administered by [redacted] daily for 25 days, which is the standard procedure that we have used in our previous studies. Mice will be checked and weighed daily until the conclusion of the experiment to monitor their health. Our previous experience with this model demonstrates that the [redacted] are well tolerated by the 129/SvEv wildtype mice, which are very amenable to handling.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery. Mice in the [redacted] on the day of the operation and each day thereafter. After 3-4 days they will be replaced into a communal cage of 2-5 animals.

Mice will recieve [redacted] on days 3, 5, and 7 post-surgery. For [redacted] will be injected into the tail vein on day 4 post-surgery. To generate [redacted] At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out.

ii) How will the level of anaesthesia be monitored.
Pedal withdrawl (toe-pinch) reflex method of choice for most species
iii) Please describe the frequency and duration of post-operative monitoring

The University of Otago expects that all animals shall be monitored continuously post-anaesthetic until they are fully ambulatory, then twice daily follwoing major survival surgery and once daily following minor survival surgery, for a minimum of 4 days. Standardised monitoring sheets are available.

Alternative monitoring arrangements may be needed when animals are released post-operatively in wildlife studies.

Frequency = number of times per day, Duration = number of days.

Answer

Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

iv) What monitoring sheets will be used?
University of Otago Animal Welfare Monitoring Sheets
v) What are the parameters to be monitored?

Body weight
Food/water intake
Level of activity
Diarrhea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Coat rough/fur on end
Hunched posture
Pain behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear Position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**

Other

Tumour size should not exceed 200 mm^2

vi) Will individual animals undergo multiple surgeries?
Yes

The first procedure is to implant the [redacted] into the fat pad. The second procedure is a biopsy of the tumour before treatment begins. It is vital to have a baseline measurement of the tumour before treatment begins so that we can measure the effect the drugs have on the tumour. An ovariectomy is also performed at the same time, and again this is vital to effectively halt production of oestrogen in the body.

vii) Will paralytic agents be administered in this study?
No
viii) Please confirm that post-operative analgesia will be routinely administered for all but minor surgeries.
Yes, analgesics will be used and details are provided in the RVM table.

5.2.10 Potential Stressors

Describe the potential stressors that animals may experience during procedures conducted on this protocol.
Answer

Isolation stress: Following the initial biopsy and ovariectomy, mice will be housed individually for 3-4 days until the wound has essentially healed and any pain has alleviated. Mice are housed within sight and sound of other mice, and returned to communal housing as soon as possible

Animal number calculation for study segment 1. [redacted]

Mouse		
	Max	Factor
	12	
	12	**Mouse**

7 Manipulations & Procedures

Description	Species
Tumour Biopsy, [redacted], and Ovariectomy	Mouse
RVM Procedures	Mouse
Survival RVM Procedure	Mouse

7.1 Description of manipulations & procedures

2. [redacted]

Species to be used for the study segment

Mouse

5.2.1 Study Segment Summary

Briefly explain the experimental design (is this a pilot or main study) and specify all animal manipulations used in this study segment.

Specific manipulations to be employed in the study must be described on the "Manipulations" tab. The Study Segment Summary should be written in an ***animal-centric*** *fashion to allow the AEC to understand the* ***experimental course of an animal from its entry into the experiment to the endpoint*** *of the study. A flowchart of the individual manipulations may be an effective presentation of the study segment. You can attach a flow chart using the attachments button at the bottom of the form.*

Answer

The following procedures will be used for the main study. To generate [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, dormitor and atropine) and a [redacted] will be injected subcutaneously into the mammary tissue. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring. Mice will be monitored at least twice weekly until tumours become palpable. Once palpable, tumours will be measured with calipers 2-3 times per week until the tumour reaches 49 mm^2. It usually takes about 20-40 days for the tumours to reach 49 mm^2

When a tumour reaches 49 mm^2, a 18-gauge core biopsy will be taken. Mice receive antibiotic (Amphoprim) prior to surgery and will be anaesthetised (Ketamine, Domitor, Atropine). An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive anaesthetic. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to staunch any bleeding and the skin sutured as required.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery, and mice will receive analgesics (Temgesic) twice daily for 3-4 days post-surgery. Mice in the [redacted] receive a [redacted] on the day of the operation and each day thereafter until day 25. After 3-4 days they will be replaced into a communal cage of 2-5 animals. Mice in the [redacted] will receive a [redacted] on the day of the operation and each day thereafter until day 25. Mice in the [redacted] on days 3, 5, and 7 post-surgery for a total of [redacted] per mouse. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out. At the end of 25 days, mice will be euthanised and a blood sample will be taken. We will collect the tumour, spleen and lymphoid tissues. The tissues obtained from these experiments will then be analysed to address the experimental objectives. Please see the attached flow chart illustration for further clarification.

5.2.2 Study Segment

Is this study segment the same as any other study segment?
Yes

If the same manipulations are used in each segment, with the same species and the same adverse effects are anticipated, then this section only needs to be completed once.

Please advise which other study segments are the same as this one.

Segments 1-6

5.2.3 Adverse Effects

A) Please indicate if any of the following adverse effects may potentially occur during the proposed study.
Weight Loss
Haemorrhage
Infection
Pain
Other

Tumour ulceration

B) If any of the above adverse effects were selected then please specify how the adverse effect will be managed.

If you have not ticked any of the above adverse effects, please enter n/a

Answer

Adverse side effects, apart from ulceration of the tumour as discussed previously, are unlikely but if a mouse meets any of the standard international humane endpoints we will euthanize the animal immediately. Pain will be well managed with drugs following the biopsy of the tumour and ovariectomy. We have not experienced haemorrhage or wound infection following ovariectomy, but are a risk in all surgeries. Careful surgical technique and administration of antibiotics reduce this to a very low risk. Some weight loss may occur as the tumour increases in size and this will be monitored by regular weighing and assessment of the overall condition of the mouse, however this has not been noticed in any mice as of yet. With any application of anaesthesia to mice, there is likely to be some small amount of weight loss, as the activity and appetite of the mouse is reduced for several hours. However, we would expect less than 10% body weight loss, and the mice will be monitored with twice daily weight measurements for 4 days following surgery. Mice quickly recover the weight loss within a few days, and in the event weight loss is greater than the humane endpoints, the mouse will be euthanized.

C) Detail the survival rates and incidence of adverse events for the proposed manipulations based on the relevant literature and your experience of the manipulations
Answer

This [redacted] has been developed within the last 5 years and, to date, only appears in limited number of papers, which unfortunately do not investigate long term survival rates. Neither paper mention ulcers or adverse effects, however personal communication with one of the authors confirms that ulceration occurred in some cases. We have a successful tumour take rate of 94%, which is similar to other mammary xenografts.

Our efforts to refine the model including reducing the [redacted] have reduced the rate of ulceration amongst our mice to below 20% (down from ~25%). Some ulcers formed quickly before the tumour was large enough to begin treatment, and others developed after the initial biopsy at any time ranging from 4 - 23 days post biopsy and commencement of treatment. Ulcers may occur for a variety of reasons, and we have addressed each of these causes in the attempt to reduce ulceration. We have confirmed the [redacted] are free from bacteria and mycoplasma, and that the [redacted] into the mammary gland, not subcutaneously. We are using the smallest volume of injection possible under sterile conditions and prepare the tumour carefully for biopsy. We have explored the possibility of using alternative, more invasive, surgical techniques to implant tumours with the aid and supervision of the [redacted] in 2014 and in November 2017 but, to date these pilot animals showed that [redacted] into the mammary fat pad was more effective using our current method. We also discussed alternative approaches with [redacted] and [redacted] recommended this method was most effective, based on the large number of animals [redacted] had used mammary fat pad implantation on. We have also discussed a change to softer bedding with [redacted]. It is known that [redacted] are more prone to ulceration than others, and unfortunately it appears [redacted] are one of these, however there are no other suitable [redacted] that we can utilise for this study.

Ovariectomy is a routine procedure carried out in many mouse facilities around the world, and [redacted] has had very high survival rates following ovariectomy in our previous study. The tumour biopsy results in minor bleeding which is easily controlled and due to the small gauge of the needle there is only a small wound in the skin which heals well. Mice will be treated with antibiotics and painkillers and we expect minimal complications following surgery. Ovariectomy can cause adverse effects over a longer time period, however given the short length of the study, we do not expect any of these to occur.

[redacted] works by [redacted], and is unlikely to have any significant effects on the mice. Some papers have reported lower uterine weights and some changes in bone density, but as mice are only exposed to [redacted] for 25 days, we have not observed any significant effects on the mice. We have investigated the possibility of an [redacted]. However, the pumps available are only able to carry a sufficient volume of [redacted] to last 50 hours so would have to be refilled every second day which we believe negates the advantage of using the pump. In addition, the mice must be handled every day regardless of whether we use [redacted]. Currently, we are able to combine the weighing, monitoring, and injection into one event. Moreover, the concentration of [redacted] would need to be increased. Previously when using a higher concentration of [redacted], we observed a local skin reaction, which we were able to eliminate by reducing the concentration and injecting a larger volume. We are concerned that with a slow release of a small volume of concentrated drug in a fixed position, that there would be a local inflammatory reaction. The subcutaneous injections allow us to rotate the position of injection to different parts of the body to avoid repeatedly irritating the same site.

Our [redacted] will be prepared according the method developed by our collaborator at the [redacted] which has previously been shown to be well tolerated by mice. In our recent study [redacted] we have encountered some problems with lack of recovery after surgery which is probably due to the timing of the [redacted] and we have changed the timing of the [redacted] to prevent this from happening in the future.

5.2.4 Animal Monitoring

A) Please describe the frequency and duration of animal monitoring over the course of the study

Frequency = number of times per day, Duration = number of days

Answer
Prior to [redacted], mice will be checked a minimum of twice weekly. Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

B) Please attach an example of the monitoring sheet to be used. Please note all sheets (including electronic records) need to be available for review by the AWO upon request.
Other (Please attach a sample to this protocol by using the attachments button at the top of the form.)

C) What are the parameters to be monitored?
Body weight
Food/water intake
Level of activity
Diarrhoea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Body Condition
Coat rough, fur on end
Hunched Posture
Pain Behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**
- **Cheek bulge**

Other
Tumour size should not exceed 200 mm^2

5.2.5 Endpoints

A) Is death an endpoint?

Death as an endpoint, which in biomedical science terms means the animal is left to die as part of the experimental protocol without any alleviation of suffering or human intervention is generally ethically unacceptable and must be fully justified.

No – animals will be killed

B) Indicate any additional humane endpoint criteria (other than those listed above) that may apply to this study.
Please include any other information you can provide on euthanasia endpoints for these animals
Tumour ulceration

C) Experimental Endpoint: What will determine the experimental endpoint(s) of the study?

For example: state the specific time points, specific tumour size, etc.

Answer
Mice will be euthanized at the end of the 25 day drug treatment, or when the tumour reaches a maximum size of 200mm2, whichever comes first.

5.2.6 Euthanasia

A) Describe the method of killing and euthanasia that will be used

Note that some methods may require scientific justification

CO2 chamber

B) Please select the method of ensuring death of the animal
Cervical Dislocation

5.2.7 Breeding

Are you breeding animals with a potential adverse phenotype under this protocol?
No

5.2.8 Animal Identification Methods

Describe method and age at which animals are identified.
Answer

Each animal will be identified with a unique cage card number. In addition, each animal inside the cage will have an ear punch unique to that cage number which allows us to identify individual mice by a unique ID number assigned to that mouse by the research group. Each cage is allowed to house a total of 5 mice, thus, the ear punches will be as follows: 1.) No Mark (NM) 2.) Left Ear (LE), 3.) Right Ear (RE), 4.) Left Ear/Right Ear (LE/RE), and Left Ear x2 (LEx2). Our system allows for the least amount of ear punches possible while still maintaining uniqueness among 5 mice. This will help reduce pain to the mice by using less ear punches. For example, if only 3 mice are in a cage the ear marks chosen will ALWAYS be 1.) NM, 2.) LE, and 3.) RE, resulting in a total of 2 ear punches total; as oppose to using 1.) LE, 2.) LE/RE, and 3.) LEx2, resulting in 5 ear punches total. We have previously tried using ear tags, but noticed that some of the mice developed a red, swollen, inflammed area at the site of the ear tag. Our study is investigating immunological responses in the animal, so the ear tags are not ideal for our studies as they may add in confounding factors regarding the immune response in the mouse. The animals will be identified at the time of subcutaneous [redacted].

5.2.9 Surgery

A) Will you be performing surgery on this study segment?
Yes

i) Will the surgery be survival or non-survival?
Survival surgery

Please describe the surgery to be performed

Please provide the following details on the surgery:

Details of the sterilisation of all equipment, implants, surgical drapes etc and of any re-sterilisation during surgery (between animals).

Details of PPE used and how it is sterilised.

Preparation of surgical site.

Full details of the surgery – including drugs used.

Details of type of ear bars used for stereotaxic surgery.

Details of implants (size and weight).

1st surgery: Tumour implantation

To generate [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, domitor and atropine) and a [redacted] injected percutaenously into the mammary tissue. The hair is removed by clippers and hair removal cream to assist in locating the nipple. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. The area is sterilized with 100% ethanol before the cells are injected. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring.

2nd surgery: Ovariectomy, biopsy and [redacted].

Mice will be anaesthetised for this procedure and at the same time, they will be ovariectomised, or receive a sham overiectomy (for those mice not receiving [redacted]), and have have their tumours biopsied. This is required to prevent any [redacted] and ensure the success of the [redacted] treatment. Mice receive analgesics (Temgesic) and antibiotics (Amphoprim) prior to surgery. An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive analgesic. Surgical instruments are autoclaved for each animal, or sterilised between animals using the Germinator 500 bead steriliser in [redacted]. The drapes are single use, sterile, clear, plastic drapes sourced from the [redacted], and gloves are sterilised with 100% ethanol. Hair is removed from the skin with a combination of clippers and hair removal cream. The skin is prepped by washing the incision area from inside to out with betadine and then 100% ethanol. A clean gown, hair net and facemask are worn. The biopsy guns are surgical grade and are also sterilized with 100% ethanol between uses. The biopsy guns are typically reused 5 times before being discarded. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. A small incision is made in the abdominal wall and the ovary and oviduct removed before closure with sterile sutures. The abdominal wall and skin are closed separately. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to stauch any bleeding and the skin sutured as required. On the following day, treatment with [redacted] or vehicle will be commenced. [redacted] (or a vehicle control) will be administered by subcutaneous injection daily for 25 days, which is the standard procedure that we have used in our previous studies. Mice will be checked and weighed daily until the conclusion of the experiment to monitor their health. Our previous experience with this model demonstrates that the daily injections are well tolerated by the 129/SvEv wildtype mice, which are very amenable to handling.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery. Mice in the [redacted] on the day of the operation and each day thereafter. After 3-4 days they will be replaced into a communal cage of 2-5 animals.

Mice will recieve [redacted] on days 3, 5, and 7 post-surgery. For [redacted] will be injected into the tail vein on day 4 post-surgery. To [redacted] At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out.

ii) How will the level of anaesthesia be monitored.
Pedal withdrawl (toe-pinch) reflex method of choice for most species
iii) Please describe the frequency and duration of post-operative monitoring

The University of Otago expects that all animals shall be monitored continuously post-anaesthetic until they are fully ambulatory, then twice daily follwoing major survival surgery and once daily following minor survival surgery, for a minimum of 4 days. Standardised monitoring sheets are available.

Alternative monitoring arrangements may be needed when animals are released post-operatively in wildlife studies.

Frequency = number of times per day, Duration = number of days.

Answer

Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

iv) What monitoring sheets will be used?
University of Otago Animal Welfare Monitoring Sheets
v) What are the parameters to be monitored?
Body weight

Food/water intake
Level of activity
Diarrhea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Coat rough/fur on end
Hunched posture
Pain behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear Position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**

Other

Tumour size should not exceed 200 mm^2

vi) Will individual animals undergo multiple surgeries?
Yes

The first procedure is to implant the [redacted] into the fat pad. The second procedure is a biopsy of the tumour before treatment begins. It is vital to have a baseline measurement of the tumour before treatment begins so that we can measure the effect the drugs have on the tumour. An ovariectomy is also performed at the same time, and again this is vital to effectively halt production of oestrogen in the body.

vii) Will paralytic agents be administered in this study?
No
viii) Please confirm that post-operative analgesia will be routinely administered for all but minor surgeries.
Yes, analgesics will be used and details are provided in the RVM table.

5.2.10 Potential Stressors

Describe the potential stressors that animals may experience during procedures conducted on this protocol.
Answer

Isolation stress: Following the initial biopsy and ovariectomy, mice will be housed individually for 3-4 days until the wound has essentially healed and any pain has alleviated. Mice are housed within sight and sound of other mice, and returned to communal housing as soon as possible

Animal number calculation for study segment 2. [redacted]

Mouse		
	Max	Factor
	12	
	12	**Mouse**

7 Manipulations & Procedures

Not applicable

7.1 Description of manipulations & procedures

3. [redacted]

Species to be used for the study segment

Mouse

5.2.1 Study Segment Summary

Briefly explain the experimental design (is this a pilot or main study) and specify all animal manipulations used in this study segment.

*Specific manipulations to be employed in the study must be described on the "Manipulations" tab. The Study Segment Summary should be written in an **animal-centric** fashion to allow the AEC to understand the **experimental course of an animal from its entry into the experiment to the endpoint** of the study. A flowchart of the individual manipulations may be an effective presentation of the study segment. You can attach a flow chart using the attachments button at the bottom of the form.*

Answer

The following procedures will be used for the main study. To [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, dormitor and atropine) and a [redacted] will be injected subcutaneously into the mammary tissue. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring. Mice will be monitored at least twice weekly until tumours become palpable. Once palpable, tumours will be measured with calipers 2-3 times per week until the tumour reaches 49 mm^2. It usually takes about 20-40 days for the tumours to reach 49 mm^2

When a tumour reaches 49 mm^2, a 18-gauge core biopsy will be taken. Mice receive antibiotic (Amphoprim) prior to surgery and will be anaesthetised (Ketamine, Dormitor, Atropine). An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive anaesthetic. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to staunch any bleeding and the skin sutured as required.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery, and mice will receive analgesics (Temgesic) twice daily for 3-4 days post-surgery. Mice in the [redacted] receive a [redacted] the day of the operation and each day thereafter until day 25. After 3-4 days they will be replaced into a communal cage of 2-5 animals. Mice in the [redacted] on the day of the operation and each day thereafter until day 25. Mice in the [redacted] on days 3, 5, and 7 post-surgery for a total of [redacted] per mouse. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out. At the end of 25 days, mice will be euthanised and a blood sample will be taken. We will collect the tumour, spleen and lymphoid tissues. The tissues obtained from these experiments will then be analysed to address the experimental objectives. Please see the attached flow chart illustration for further clarification.

5.2.2 Study Segment

Is this study segment the same as any other study segment?
Yes

If the same manipulations are used in each segment, with the same species and the same adverse effects are anticipated, then this section only needs to be completed once.

Please advise which other study segments are the same as this one.

Segments 1-6

5.2.3 Adverse Effects

A) Please indicate if any of the following adverse effects may potentially occur during the proposed study.
Weight Loss
Haemorrhage
Infection
Pain
Other

Tumour ulceration

B) If any of the above adverse effects were selected then please specify how the adverse effect will be managed.

If you have not ticked any of the above adverse effects, please enter n/a

Answer

Adverse side effects, apart from ulceration of the tumour as discussed previously, are unlikely but if a mouse meets any of the standard international humane endpoints we will euthanize the animal immediately. Pain will be well managed with drugs following the biopsy of the tumour and ovariectomy. We have not experienced haemorrhage or wound infection following ovariectomy, but are a risk in all surgeries. Careful surgical technique and administration of antibiotics reduce this to a very low risk. Some weight loss may occur as the tumour increases in size and this will be monitored by regular weighing and assessment of the overall condition of the mouse, however this has not been noticed in any mice as of yet. With any application of anaesthesia to mice, there is likely to be some small amount of weight loss, as the activity and appetite of the mouse is reduced for several hours. However, we would expect less than 10% body weight loss, and the mice will be monitored with twice daily weight measurements for 4 days following surgery. Mice quickly recover the weight loss within a few days, and in the event weight loss is greater than the humane endpoints, the mouse will be euthanized.

C) Detail the survival rates and incidence of adverse events for the proposed manipulations based on the relevant literature and your experience of the manipulations
Answer

This [redacted] has been developed within the last 5 years and, to date, only appears in limited number of papers, which unfortunately do not investigate long term survival rates. Neither paper mention ulcers or adverse effects, however personal communication with one of the authors confirms that ulceration occurred in some cases. We have a successful tumour take rate of 94%, which is similar to other mammary xenografts.

Our efforts to refine the model including reducing the [redacted] have reduced the rate of ulceration amongst our mice to below 20% (down from ~25%). Some ulcers formed quickly before the tumour was large enough to begin treatment, and others developed after the initial biopsy at any time ranging from 4 - 23 days post biopsy and commencement of treatment. Ulcers may occur for a variety of reasons, and we have addressed each of these causes in the attempt to reduce ulceration. We have confirmed the [redacted] are free from bacteria and mycoplasma, and that the [redacted] into the mammary gland, not subcutaneously. We are using the smallest volume of injection possible under sterile conditions and prepare the tumour carefully for biopsy. We have explored the possibility of using alternative, more invasive, surgical techniques to implant tumours with the aid and supervision of the [redacted] in 2014 and in November 2017 but, to date these pilot animals showed that [redacted] into the mammary fat pad was more effective using our current method. We also discussed alternative approaches with [redacted] and [redacted] recommended this method was most effective, based on the large number of animals [redacted] had used mammary fat pad implantation on. We have also discussed a change to softer bedding with [redacted]. It is known that some [redacted] are more prone to ulceration than others, and unfortunately it appears [redacted] are one of these, however there are no other suitable [redacted] that we can utilise for this study.

Ovariectomy is a routine procedure carried out in many mouse facilities around the world, and [redacted] has had very high survival rates following ovariectomy in our previous study. The tumour biopsy results in minor bleeding which is easily controlled and due to the small gauge of the needle there is only a small wound in the skin which heals well. Mice will be treated with antibiotics and painkillers and we expect minimal complications following surgery. Ovariectomy can cause adverse effects over a longer time period, however given the short length of the study, we do not expect any of these to occur.

[redacted] works by lowering the production of [redacted], and is unlikely to have any significant effects on the mice. Some papers have reported lower uterine weights and some changes in bone density, but as mice are only exposed to [redacted] for 25 days, we have not observed any significant effects on the mice. We have investigated the possibility of an [redacted] to deliver [redacted]. However, the pumps available are only able to carry a sufficient volume of [redacted] to last 50 hours so would have to be refilled every second day which we believe negates the advantage of using the pump. In addition, the mice must be handled every day regardless of whether we use [redacted]. Currently, we are able to combine the weighing, monitoring, and injection into one event. Moreover, the concentration of [redacted] would need to be increased. Previously when using a higher concentration of [redacted], we observed a local skin reaction, which we were able to eliminate by reducing the concentration and injecting a larger volume. We are concerned that with a slow release of a small volume of concentrated drug in a fixed position, that there would be a local inflammatory reaction. The subcutaneous injections allow us to rotate the position of injection to different parts of the body to avoid repeatedly irritating the same site.

Our [redacted] will be prepared according the method developed by our collaborator at the [redacted] which has previously been shown to be well tolerated by mice. In our recent study [redacted] we have encountered some problems with lack of recovery after surgery which is probably due to the timing of the [redacted] and we have changed the timing of the [redacted] to prevent this from happening in the future.

5.2.4 Animal Monitoring

A) Please describe the frequency and duration of animal monitoring over the course of the study

Frequency = number of times per day, Duration = number of days

Answer
Prior to [redacted] mice will be checked a minimum of twice weekly. Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

B) Please attach an example of the monitoring sheet to be used. Please note all sheets (including electronic records) need to be available for review by the AWO upon request.
Other (Please attach a sample to this protocol by using the attachments button at the top of the form.)

C) What are the parameters to be monitored?
Body weight
Food/water intake
Level of activity
Diarrhoea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Body Condition
Coat rough, fur on end
Hunched Posture
Pain Behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**
- **Cheek bulge**

Other
Tumour size should not exceed 200 mm^2

5.2.5 Endpoints

A) Is death an endpoint?

Death as an endpoint, which in biomedical science terms means the animal is left to die as part of the experimental protocol without any alleviation of suffering or human intervention is generally ethically unacceptable and must be fully justified.

No – animals will be killed

B) Indicate any additional humane endpoint criteria (other than those listed above) that may apply to this study.
Please include any other information you can provide on euthanasia endpoints for these animals
Tumour ulceration

C) Experimental Endpoint: What will determine the experimental endpoint(s) of the study?

For example: state the specific time points, specific tumour size, etc.

Answer
Mice will be euthanized at the end of the 25 day drug treatment, or when the tumour reaches a maximum size of 200mm2, whichever comes first.

5.2.6 Euthanasia

A) Describe the method of killing and euthanasia that will be used

Note that some methods may require scientific justification

CO2 chamber

B) Please select the method of ensuring death of the animal
Cervical Dislocation

5.2.7 Breeding

Are you breeding animals with a potential adverse phenotype under this protocol?
No

5.2.8 Animal Identification Methods

Describe method and age at which animals are identified.
Answer

Each animal will be identified with a unique cage card number. In addition, each animal inside the cage will have an ear punch unique to that cage number which allows us to identify individual mice by a unique ID number assigned to that mouse by the research group. Each cage is allowed to house a total of 5 mice, thus, the ear punches will be as follows: 1.) No Mark (NM) 2.) Left Ear (LE), 3.) Right Ear (RE), 4.) Left Ear/Right Ear (LE/RE), and Left Ear x2 (LEx2). Our system allows for the least amount of ear punches possible while still maintaining uniqueness among 5 mice. This will help reduce pain to the mice by using less ear punches. For example, if only 3 mice are in a cage the ear marks chosen will ALWAYS be 1.) NM, 2.) LE, and 3.) RE, resulting in a total of 2 ear punches total; as oppose to using 1.) LE, 2.) LE/RE, and 3.) LEx2, resulting in 5 ear punches total. We have previously tried using ear tags, but noticed that some of the mice developed a red, swollen, inflammed area at the site of the ear tag. Our study is investigating immunological responses in the animal, so the ear tags are not ideal for our studies as they may add in confounding factors regarding the immune response in the mouse. The animals will be identified at the time of subcutaneous [redacted].

5.2.9 Surgery

A) Will you be performing surgery on this study segment?
Yes

i) Will the surgery be survival or non-survival?
Survival surgery

Please describe the surgery to be performed

Please provide the following details on the surgery:

Details of the sterilisation of all equipment, implants, surgical drapes etc and of any re-sterilisation during surgery (between animals).

Details of PPE used and how it is sterilised.

Preparation of surgical site.

Full details of the surgery – including drugs used.

Details of type of ear bars used for stereotaxic surgery.

Details of implants (size and weight).

1st surgery: Tumour implantation

To generate [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, domitor and atropine) and a [redacted] injected percutaenously into the mammary tissue. The hair is removed by clippers and hair removal cream to assist in locating the nipple. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. The area is sterilized with 100% ethanol before the cells are injected. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring.

2nd surgery: Ovariectomy, biopsy and [redacted].

Mice will be anaesthetised for this procedure and at the same time, they will be ovariectomised, or receive a sham overiectomy (for those mice not receiving [redacted]), and have have their tumours biopsied. This is required to prevent any [redacted] production and ensure the success of the [redacted] treatment. Mice receive analgesics (Temgesic) and antibiotics (Amphoprim) prior to surgery. An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive analgesic. Surgical instruments are autoclaved for each animal, or sterilised between animals using the Germinator 500 bead steriliser in [redacted]. The drapes are single use, sterile, clear, plastic drapes sourced from the [redacted], and gloves are sterilised with 100% ethanol. Hair is removed from the skin with a combination of clippers and hair removal cream. The skin is prepped by washing the incision area from inside to out with betadine and then 100% ethanol. A clean gown, hair net and facemask are worn. The biopsy guns are surgical grade and are also sterilized with 100% ethanol between uses. The biopsy guns are typically reused 5 times before being discarded. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. A small incision is made in the abdominal wall and the ovary and oviduct removed before closure with sterile sutures. The abdominal wall and skin are closed separately. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to stauch any bleeding and the skin sutured as required. On the following day, treatment with [redacted] will be commenced. [redacted]) will be administered by subcutaneous injection daily for 25 days, which is the standard procedure that we have used in our previous studies. Mice will be checked and weighed daily until the conclusion of the experiment to monitor their health. Our previous experience with this model demonstrates that the daily injections are well tolerated by the 129/SvEv wildtype mice, which are very amenable to handling.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery. Mice in the [redacted] on the day of the operation and each day thereafter. After 3-4 days they will be replaced into a communal cage of 2-5 animals.

Mice will recieve [redacted] on days 3, 5, and 7 post-surgery. For [redacted] will be injected into the tail vein on day 4 post-surgery. To [redacted] The [redacted] At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out.

ii) How will the level of anaesthesia be monitored.
Pedal withdrawl (toe-pinch) reflex method of choice for most species
iii) Please describe the frequency and duration of post-operative monitoring

The University of Otago expects that all animals shall be monitored continuously post-anaesthetic until they are fully ambulatory, then twice daily follwoing major survival surgery and once daily following minor survival surgery, for a minimum of 4 days. Standardised monitoring sheets are available.

Alternative monitoring arrangements may be needed when animals are released post-operatively in wildlife studies.

Frequency = number of times per day, Duration = number of days.

Answer

Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

iv) What monitoring sheets will be used?
University of Otago Animal Welfare Monitoring Sheets
v) What are the parameters to be monitored?
Body weight

Food/water intake
Level of activity
Diarrhea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Coat rough/fur on end
Hunched posture
Pain behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear Position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**

Other

Tumour size should not exceed 200 mm^2

vi) Will individual animals undergo multiple surgeries?
Yes

The first procedure is to [redacted] into the fat pad. The second procedure is a biopsy of the tumour before treatment begins. It is vital to have a baseline measurement of the tumour before treatment begins so that we can measure the effect the drugs have on the tumour. An ovariectomy is also performed at the same time, and again this is vital to effectively halt production of [redacted] in the body.

vii) Will paralytic agents be administered in this study?
No
viii) Please confirm that post-operative analgesia will be routinely administered for all but minor surgeries.
Yes, analgesics will be used and details are provided in the RVM table.

5.2.10 Potential Stressors

Describe the potential stressors that animals may experience during procedures conducted on this protocol.
Answer

Isolation stress: Following the initial biopsy and ovariectomy, mice will be housed individually for 3-4 days until the wound has essentially healed and any pain has alleviated. Mice are housed within sight and sound of other mice, and returned to communal housing as soon as possible

Animal number calculation for study segment 3. [redacted]

Mouse		
	Max	Factor
	12	
	12	**Mouse**

7 Manipulations & Procedures

Not applicable

7.1 Description of manipulations & procedures

4. [redacted]

Species to be used for the study segment

Mouse

5.2.1 Study Segment Summary

Briefly explain the experimental design (is this a pilot or main study) and specify all animal manipulations used in this study segment.

*Specific manipulations to be employed in the study must be described on the "Manipulations" tab. The Study Segment Summary should be written in an **animal-centric** fashion to allow the AEC to understand the **experimental course of an animal from its entry into the experiment to the endpoint** of the study. A flowchart of the individual manipulations may be an effective presentation of the study segment. You can attach a flow chart using the attachments button at the bottom of the form.*

Answer

The following procedures will be used for the main study. To [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, dormitor and atropine) and a [redacted] will be injected subcutaneously into the mammary tissue. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring. Mice will be monitored at least twice weekly until tumours become palpable. Once palpable, tumours will be measured with calipers 2-3 times per week until the tumour reaches 49 mm^2. It usually takes about 20-40 days for the tumours to reach 49 mm^2

When a tumour reaches 49 mm^2, a 18-gauge core biopsy will be taken. Mice receive antibiotic (Amphoprim) prior to surgery and will be anaesthetised (Ketamine, Domitor, Atropine). An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive anaesthetic. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to staunch any bleeding and the skin sutured as required.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery, and mice will receive analgesics (Temgesic) twice daily for 3-4 days post-surgery. Mice in the [redacted] receive a [redacted] on the day of the operation and each day thereafter until day 25. After 3-4 days they will be replaced into a communal cage of 2-5 animals. Mice in the [redacted] n the day of the operation and each day thereafter until day 25. Mice in the [redacted] on days 3, 5, and 7 post-surgery for a total of [redacted] per mouse. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out. At the end of 25 days, mice will be euthanised and a blood sample will be taken. We will collect the tumour, spleen and lymphoid tissues. The tissues obtained from these experiments will then be analysed to address the experimental objectives. Please see the attached flow chart illustration for further clarification.

5.2.2 Study Segment

Is this study segment the same as any other study segment?
Yes

If the same manipulations are used in each segment, with the same species and the same adverse effects are anticipated, then this section only needs to be completed once.

Please advise which other study segments are the same as this one.

Segments 1-6

5.2.3 Adverse Effects

A) Please indicate if any of the following adverse effects may potentially occur during the proposed study.
Weight Loss
Haemorrhage
Infection
Pain
Other

Tumour ulceration

B) If any of the above adverse effects were selected then please specify how the adverse effect will be managed.

If you have not ticked any of the above adverse effects, please enter n/a

Answer

Adverse side effects, apart from ulceration of the tumour as discussed previously, are unlikely but if a mouse meets any of the standard international humane endpoints we will euthanize the animal immediately. Pain will be well managed with drugs following the biopsy of the tumour and ovariectomy. We have not experienced haemorrhage or wound infection following ovariectomy, but are a risk in all surgeries. Careful surgical technique and administration of antibiotics reduce this to a very low risk. Some weight loss may occur as the tumour increases in size and this will be monitored by regular weighing and assessment of the overall condition of the mouse, however this has not been noticed in any mice as of yet. With any application of anaesthesia to mice, there is likely to be some small amount of weight loss, as the activity and appetite of the mouse is reduced for several hours. However, we would expect less than 10% body weight loss, and the mice will be monitored with twice daily weight measurements for 4 days following surgery. Mice quickly recover the weight loss within a few days, and in the event weight loss is greater than the humane endpoints, the mouse will be euthanized.

C) Detail the survival rates and incidence of adverse events for the proposed manipulations based on the relevant literature and your experience of the manipulations
Answer

This [redacted] has been developed within the last 5 years and, to date, only appears in limited number of papers, which unfortunately do not investigate long term survival rates. Neither paper mention ulcers or adverse effects, however personal communication with one of the authors confirms that ulceration occurred in some cases. We have a successful tumour take rate of 94%, which is similar to other mammary xenografts.

Our efforts to refine the model including reducing the [redacted] injected have reduced the rate of ulceration amongst our mice to below 20% (down from ~25%). Some ulcers formed quickly before the tumour was large enough to begin treatment, and others developed after the initial biopsy at any time ranging from 4 - 23 days post biopsy and commencement of treatment. Ulcers may occur for a variety of reasons, and we have addressed each of these causes in the attempt to reduce ulceration. We have confirmed the [redacted] are free from bacteria and mycoplasma, and that the [redacted] injected into the mammary gland, not subcutaneously. We are using the smallest volume of injection possible under sterile conditions and prepare the tumour carefully for biopsy. We have explored the possibility of using alternative, more invasive, surgical techniques to [redacted] with the aid and supervision of the [redacted] in 2014 and in November 2017 but, to date these pilot animals showed that [redacted] into the mammary fat pad was more effective using our current method. We also discussed alternative approaches with [redacted] and [redacted] recommended this method was most effective, based on the large number of animals [redacted] had used mammary fat pad implantation on. We have also discussed a change to softer bedding with [redacted]. It is known that [redacted] are more prone to ulceration than others, and unfortunately it appears [redacted] are one of these, however there are no other suitable [redacted] that we can utilise for this study.

Ovariectomy is a routine procedure carried out in many mouse facilities around the world, and [redacted] has had very high survival rates following ovariectomy in our previous study. The tumour biopsy results in minor bleeding which is easily controlled and due to the small gauge of the needle there is only a small wound in the skin which heals well. Mice will be treated with antibiotics and painkillers and we expect minimal complications following surgery. Ovariectomy can cause adverse effects over a longer time period, however given the short length of the study, we do not expect any of these to occur.

[redacted] works by lowering the production of oestrogen, and is unlikely to have any significant effects on the mice. Some papers have reported lower uterine weights and some changes in bone density, but as mice are only exposed to [redacted] for 25 days, we have not observed any significant effects on the mice. We have investigated the possibility of an [redacted] to deliver [redacted]. However, the pumps available are only able to carry a sufficient volume of [redacted] to last 50 hours so would have to be refilled every second day which we believe negates the advantage of using the pump. In addition, the mice must be handled every day regardless of whether we use s.c injections or a pump. Currently, we are able to combine the weighing, monitoring, and injection into one event. Moreover, the concentration of [redacted] would need to be increased. Previously when using a higher concentration of [redacted] we observed a local skin reaction, which we were able to eliminate by reducing the concentration and injecting a larger volume. We are concerned that with a slow release of a small volume of concentrated drug in a fixed position, that there would be a local inflammatory reaction. The subcutaneous injections allow us to rotate the position of injection to different parts of the body to avoid repeatedly irritating the same site.

Our [redacted] will be prepared according the method developed by our collaborator at the [redacted] which has previously been shown to be well tolerated by mice. In our recent study [redacted] we have encountered some problems with lack of recovery after surgery which is probably due to the timing of the [redacted] and we have changed the timing of the [redacted] to prevent this from happening in the future.

5.2.4 Animal Monitoring

A) Please describe the frequency and duration of animal monitoring over the course of the study

Frequency = number of times per day, Duration = number of days

Answer
Prior to [redacted], mice will be checked a minimum of twice weekly. Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

B) Please attach an example of the monitoring sheet to be used. Please note all sheets (including electronic records) need to be available for review by the AWO upon request.
Other (Please attach a sample to this protocol by using the attachments button at the top of the form.)

C) What are the parameters to be monitored?
Body weight
Food/water intake
Level of activity
Diarrhoea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Body Condition
Coat rough, fur on end
Hunched Posture
Pain Behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**
- **Cheek bulge**

Other
Tumour size should not exceed 200 mm^2

5.2.5 Endpoints

A) Is death an endpoint?

Death as an endpoint, which in biomedical science terms means the animal is left to die as part of the experimental protocol without any alleviation of suffering or human intervention is generally ethically unacceptable and must be fully justified.

No – animals will be killed

B) Indicate any additional humane endpoint criteria (other than those listed above) that may apply to this study.
Please include any other information you can provide on euthanasia endpoints for these animals
Tumour ulceration

C) Experimental Endpoint: What will determine the experimental endpoint(s) of the study?

For example: state the specific time points, specific tumour size, etc.

Answer
Mice will be euthanized at the end of the 25 day drug treatment, or when the tumour reaches a maximum size of 200mm2, whichever comes first.

5.2.6 Euthanasia

A) Describe the method of killing and euthanasia that will be used

Note that some methods may require scientific justification

CO2 chamber

B) Please select the method of ensuring death of the animal
Cervical Dislocation

5.2.7 Breeding

Are you breeding animals with a potential adverse phenotype under this protocol?
No

5.2.8 Animal Identification Methods

Describe method and age at which animals are identified.
Answer

Each animal will be identified with a unique cage card number. In addition, each animal inside the cage will have an ear punch unique to that cage number which allows us to identify individual mice by a unique ID number assigned to that mouse by the research group. Each cage is allowed to house a total of 5 mice, thus, the ear punches will be as follows: 1.) No Mark (NM) 2.) Left Ear (LE), 3.) Right Ear (RE), 4.) Left Ear/Right Ear (LE/RE), and Left Ear x2 (LEx2). Our system allows for the least amount of ear punches possible while still maintaining uniqueness among 5 mice. This will help reduce pain to the mice by using less ear punches. For example, if only 3 mice are in a cage the ear marks chosen will ALWAYS be 1.) NM, 2.) LE, and 3.) RE, resulting in a total of 2 ear punches total; as oppose to using 1.) LE, 2.) LE/RE, and 3.) LEx2, resulting in 5 ear punches total. We have previously tried using ear tags, but noticed that some of the mice developed a red, swollen, inflammed area at the site of the ear tag. Our study is investigating immunological responses in the animal, so the ear tags are not ideal for our studies as they may add in confounding factors regarding the immune response in the mouse. The animals will be identified at the time of subcutaneous [redacted].

5.2.9 Surgery

A) Will you be performing surgery on this study segment?
Yes

i) Will the surgery be survival or non-survival?
Survival surgery

Please describe the surgery to be performed

Please provide the following details on the surgery:

Details of the sterilisation of all equipment, implants, surgical drapes etc and of any re-sterilisation during surgery (between animals).

Details of PPE used and how it is sterilised.

Preparation of surgical site.

Full details of the surgery – including drugs used.

Details of type of ear bars used for stereotaxic surgery.

Details of implants (size and weight).

1st surgery: Tumour implantation

To generate [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, domitor and atropine) and a [redacted] injected percutaenously into the mammary tissue. The hair is removed by clippers and hair removal cream to assist in locating the nipple. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. The area is sterilized with 100% ethanol before the cells are injected. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring.

2nd surgery: Ovariectomy, biopsy and [redacted].

Mice will be anaesthetised for this procedure and at the same time, they will be ovariectomised, or receive a sham overiectomy (for those mice not receiving [redacted]), and have have their tumours biopsied. This is required to prevent any [redacted] and ensure the success of the [redacted] treatment. Mice receive analgesics (Temgesic) and antibiotics (Amphoprim) prior to surgery. An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive analgesic. Surgical instruments are autoclaved for each animal, or sterilised between animals using the Germinator 500 bead steriliser in room [redacted]. The drapes are single use, sterile, clear, plastic drapes sourced from the [redacted], and gloves are sterilised with 100% ethanol. Hair is removed from the skin with a combination of clippers and hair removal cream. The skin is prepped by washing the incision area from inside to out with betadine and then 100% ethanol. A clean gown, hair net and facemask are worn. The biopsy guns are surgical grade and are also sterilized with 100% ethanol between uses. The biopsy guns are typically reused 5 times before being discarded. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. A small incision is made in the abdominal wall and the ovary and oviduct removed before closure with sterile sutures. The abdominal wall and skin are closed separately. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to stauch any bleeding and the skin sutured as required. On the following day, treatment with [redacted] or vehicle will be commenced. [redacted] will be administered by subcutaneous injection daily for 25 days, which is the standard procedure that we have used in our previous studies. Mice will be checked and weighed daily until the conclusion of the experiment to monitor their health. Our previous experience with this model demonstrates that the daily injections are well tolerated by the 129/SvEv wildtype mice, which are very amenable to handling.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery. Mice in the [redacted] on the day of the operation and each day thereafter. After 3-4 days they will be replaced into a communal cage of 2-5 animals.

Mice will recieve [redacted] on days 3, 5, and 7 post-surgery. For [redacted] will be injected into the tail vein on day 4 post-surgery. To [redacted] At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out.

ii) How will the level of anaesthesia be monitored.
Pedal withdrawl (toe-pinch) reflex method of choice for most species
iii) Please describe the frequency and duration of post-operative monitoring

The University of Otago expects that all animals shall be monitored continuously post-anaesthetic until they are fully ambulatory, then twice daily follwoing major survival surgery and once daily following minor survival surgery, for a minimum of 4 days. Standardised monitoring sheets are available.

Alternative monitoring arrangements may be needed when animals are released post-operatively in wildlife studies.

Frequency = number of times per day, Duration = number of days.

Answer

Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

iv) What monitoring sheets will be used?
University of Otago Animal Welfare Monitoring Sheets
v) What are the parameters to be monitored?
Body weight

Food/water intake
Level of activity
Diarrhea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Coat rough/fur on end
Hunched posture
Pain behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear Position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**

Other

Tumour size should not exceed 200 mm^2

vi) Will individual animals undergo multiple surgeries?
Yes

The first procedure is to implant the [redacted] into the fat pad. The second procedure is a biopsy of the tumour before treatment begins. It is vital to have a baseline measurement of the tumour before treatment begins so that we can measure the effect the drugs have on the tumour. An ovariectomy is also performed at the same time, and again this is vital to effectively halt production of [redacted] in the body.

vii) Will paralytic agents be administered in this study?
No
viii) Please confirm that post-operative analgesia will be routinely administered for all but minor surgeries.
Yes, analgesics will be used and details are provided in the RVM table.

5.2.10 Potential Stressors

Describe the potential stressors that animals may experience during procedures conducted on this protocol.
Answer

Isolation stress: Following the initial biopsy and ovariectomy, mice will be housed individually for 3-4 days until the wound has essentially healed and any pain has alleviated. Mice are housed within sight and sound of other mice, and returned to communal housing as soon as possible

Animal number calculation for study segment 4. [redacted]

Mouse		
	Max	Factor
	12	
	12	**Mouse**

7 Manipulations & Procedures

Not applicable

7.1 Description of manipulations & procedures

5. [redacted]

Species to be used for the study segment

Mouse

5.2.1 Study Segment Summary

Briefly explain the experimental design (is this a pilot or main study) and specify all animal manipulations used in this study segment.

Specific manipulations to be employed in the study must be described on the "Manipulations" tab. The Study Segment Summary should be written in an ***animal-centric*** *fashion to allow the AEC to understand the* ***experimental course of an animal from its entry into the experiment to the endpoint*** *of the study. A flowchart of the individual manipulations may be an effective presentation of the study segment. You can attach a flow chart using the attachments button at the bottom of the form.*

Answer

The following procedures will be used for the main study. To [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, dormitor and atropine) and a [redacted] will be injected subcutaneously into the mammary tissue. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring. Mice will be monitored at least twice weekly until tumours become palpable. Once palpable, tumours will be measured with calipers 2-3 times per week until the tumour reaches 49 mm^2. It usually takes about 20-40 days for the tumours to reach 49 mm^2

When a tumour reaches 49 mm^2, a 18-gauge core biopsy will be taken. Mice receive antibiotic (Amphoprim) prior to surgery and will be anaesthetised (Ketamine, Domitor, Atropine). An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive anaesthetic. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to staunch any bleeding and the skin sutured as required.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery, and mice will receive analgesics (Temgesic) twice daily for 3-4 days post-surgery. Mice in the [redacted] receive a [redacted] on the day of the operation and each day thereafter until day 25. After 3-4 days they will be replaced into a communal cage of 2-5 animals. Mice in the [redacted] on the day of the operation and each day thereafter until day 25. Mice in the [redacted] on days 3, 5, and 7 post-surgery for a total of [redacted] per mouse. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out. At the end of 25 days, mice will be euthanised and a blood sample will be taken. We will collect the tumour, spleen and lymphoid tissues. The tissues obtained from these experiments will then be analysed to address the experimental objectives. Please see the attached flow chart illustration for further clarification.

5.2.2 Study Segment

Is this study segment the same as any other study segment?
Yes

If the same manipulations are used in each segment, with the same species and the same adverse effects are anticipated, then this section only needs to be completed once.

Please advise which other study segments are the same as this one.

Segments 1-6

5.2.3 Adverse Effects

A) Please indicate if any of the following adverse effects may potentially occur during the proposed study.
Weight Loss
Haemorrhage
Infection
Pain
Other

Tumour ulceration

B) If any of the above adverse effects were selected then please specify how the adverse effect will be managed.

If you have not ticked any of the above adverse effects, please enter n/a

Answer

Adverse side effects, apart from ulceration of the tumour as discussed previously, are unlikely but if a mouse meets any of the standard international humane endpoints we will euthanize the animal immediately. Pain will be well managed with drugs following the biopsy of the tumour and ovariectomy. We have not experienced haemorrhage or wound infection following ovariectomy, but are a risk in all surgeries. Careful surgical technique and administration of antibiotics reduce this to a very low risk. Some weight loss may occur as the tumour increases in size and this will be monitored by regular weighing and assessment of the overall condition of the mouse, however this has not been noticed in any mice as of yet. With any application of anaesthesia to mice, there is likely to be some small amount of weight loss, as the activity and appetite of the mouse is reduced for several hours. However, we would expect less than 10% body weight loss, and the mice will be monitored with twice daily weight measurements for 4 days following surgery. Mice quickly recover the weight loss within a few days, and in the event weight loss is greater than the humane endpoints, the mouse will be euthanized.

C) Detail the survival rates and incidence of adverse events for the proposed manipulations based on the relevant literature and your experience of the manipulations
Answer

This [redacted] has been developed within the last 5 years and, to date, only appears in limited number of papers, which unfortunately do not investigate long term survival rates. Neither paper mention ulcers or adverse effects, however personal communication with one of the authors confirms that ulceration occurred in some cases. We have a successful tumour take rate of 94%, which is similar to other mammary xenografts.

Our efforts to refine the model including reducing the number of cells injected have reduced the rate of ulceration amongst our mice to below 20% (down from ~25%). Some ulcers formed quickly before the tumour was large enough to begin treatment, and others developed after the initial biopsy at any time ranging from 4 - 23 days post biopsy and commencement of treatment. Ulcers may occur for a variety of reasons, and we have addressed each of these causes in the attempt to reduce ulceration. We have confirmed the [redacted] are free from bacteria and mycoplasma, and that the [redacted] into the mammary gland, not subcutaneously. We are using the smallest volume of injection possible under sterile conditions and prepare the tumour carefully for biopsy. We have explored the possibility of using alternative, more invasive, surgical techniques to implant tumours with the aid and supervision of the [redacted] in 2014 and in November 2017 but, to date these pilot animals showed that [redacted] into the mammary fat pad was more effective using our current method. We also discussed alternative approaches with [redacted] and [redacted] recommended this method was most effective, based on the large number of animals [redacted] had used mammary fat pad implantation on. We have also discussed a change to softer bedding with [redacted]. It is known that some [redacted] are more prone to ulceration than others, and unfortunately it appears [redacted] are one of these, however there are no other suitable [redacted] that we can utilise for this study.

Ovariectomy is a routine procedure carried out in many mouse facilities around the world, and [redacted] has had very high survival rates following ovariectomy in our previous study. The tumour biopsy results in minor bleeding which is easily controlled and due to the small gauge of the needle there is only a small wound in the skin which heals well. Mice will be treated with antibiotics and painkillers and we expect minimal complications following surgery. Ovariectomy can cause adverse effects over a longer time period, however given the short length of the study, we do not expect any of these to occur.

[redacted] works by lowering the production of [redacted], and is unlikely to have any significant effects on the mice. Some papers have reported lower uterine weights and some changes in bone density, but as mice are only exposed to [redacted] for 25 days, we have not observed any significant effects on the mice. We have investigated the possibility of ar[redacted] to deliver[redacted]. However, the pumps available are only able to carry a sufficient volume of [redacted] to last 50 hours so would have to be refilled every second day which we believe negates the advantage of using the pump. In addition, the mice must be handled every day regardless of whether we us[redacted]. Currently, we are able to combine the weighing, monitoring, and injection into one event. Moreover, the concentration of [redacted] would need to be increased. Previously when using a higher concentration of [redacted], we observed a local skin reaction, which we were able to eliminate by reducing the concentration and injecting a larger volume. We are concerned that with a slow release of a small volume of concentrated drug in a fixed position, that there would be a local inflammatory reaction. The subcutaneous injections allow us to rotate the position of injection to different parts of the body to avoid repeatedly irritating the same site.

Our [redacted] will be prepared according the method developed by our collaborator at th[redacted] which has previously been shown to be well tolerated by mice. In our recent study [redacted] we have encountered some problems with lack of recovery after surgery which is probably due to the timing of the [redacted] and we have changed the timing of the [redacted] to prevent this from happening in the future.

5.2.4 Animal Monitoring

A) Please describe the frequency and duration of animal monitoring over the course of the study

Frequency = number of times per day, Duration = number of days

Answer
Prior to [redacted], mice will be checked a minimum of twice weekly. Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

B) Please attach an example of the monitoring sheet to be used. Please note all sheets (including electronic records) need to be available for review by the AWO upon request.
Other (Please attach a sample to this protocol by using the attachments button at the top of the form.)

C) What are the parameters to be monitored?
Body weight
Food/water intake
Level of activity
Diarrhoea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Body Condition
Coat rough, fur on end
Hunched Posture
Pain Behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**
- **Cheek bulge**

Other
Tumour size should not exceed 200 mm^2

5.2.5 Endpoints

A) Is death an endpoint?

Death as an endpoint, which in biomedical science terms means the animal is left to die as part of the experimental protocol without any alleviation of suffering or human intervention is generally ethically unacceptable and must be fully justified.

No – animals will be killed

B) Indicate any additional humane endpoint criteria (other than those listed above) that may apply to this study.
Please include any other information you can provide on euthanasia endpoints for these animals
Tumour ulceration

C) Experimental Endpoint: What will determine the experimental endpoint(s) of the study?

For example: state the specific time points, specific tumour size, etc.

Answer
Mice will be euthanized at the end of the 25 day drug treatment, or when the tumour reaches a maximum size of 200mm2, whichever comes first.

5.2.6 Euthanasia

A) Describe the method of killing and euthanasia that will be used

Note that some methods may require scientific justification

CO2 chamber

B) Please select the method of ensuring death of the animal
Cervical Dislocation

5.2.7 Breeding

Are you breeding animals with a potential adverse phenotype under this protocol?
No

5.2.8 Animal Identification Methods

Describe method and age at which animals are identified.
Answer

Each animal will be identified with a unique cage card number. In addition, each animal inside the cage will have an ear punch unique to that cage number which allows us to identify individual mice by a unique ID number assigned to that mouse by the research group. Each cage is allowed to house a total of 5 mice, thus, the ear punches will be as follows: 1.) No Mark (NM) 2.) Left Ear (LE), 3.) Right Ear (RE), 4.) Left Ear/Right Ear (LE/RE), and Left Ear x2 (LEx2). Our system allows for the least amount of ear punches possible while still maintaining uniqueness among 5 mice. This will help reduce pain to the mice by using less ear punches. For example, if only 3 mice are in a cage the ear marks chosen will ALWAYS be 1.) NM, 2.) LE, and 3.) RE, resulting in a total of 2 ear punches total; as oppose to using 1.) LE, 2.) LE/RE, and 3.) LEx2, resulting in 5 ear punches total. We have previously tried using ear tags, but noticed that some of the mice developed a red, swollen, inflammed area at the site of the ear tag. Our study is investigating immunological responses in the animal, so the ear tags are not ideal for our studies as they may add in confounding factors regarding the immune response in the mouse. The animals will be identified at the time of subcutaneous [redacted].

5.2.9 Surgery

A) Will you be performing surgery on this study segment?
Yes

i) Will the surgery be survival or non-survival?
Survival surgery

Please describe the surgery to be performed

Please provide the following details on the surgery:

Details of the sterilisation of all equipment, implants, surgical drapes etc and of any re-sterilisation during surgery (between animals).

Details of PPE used and how it is sterilised.

Preparation of surgical site.

Full details of the surgery – including drugs used.

Details of type of ear bars used for stereotaxic surgery.

Details of implants (size and weight).

1st surgery: Tumour implantation

To generate [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, domitor and atropine) and a [redacted] injected percutaenously into the mammary tissue. The hair is removed by clippers and hair removal cream to assist in locating the nipple. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. The area is sterilized with 100% ethanol before the cells are injected. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring.

2nd surgery: Ovariectomy, biopsy and [redacted].

Mice will be anaesthetised for this procedure and at the same time, they will be ovariectomised, or receive a sham overiectomy (for those mice not receiving [redacted]), and have have their tumours biopsied. This is required to prevent any [redacted] and ensure the success of the [redacted]. Mice receive analgesics (Temgesic) and antibiotics (Amphoprim) prior to surgery. An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive analgesic. Surgical instruments are autoclaved for each animal, or sterilised between animals using the Germinator 500 bead steriliser in [redacted] The drapes are single use, sterile, clear, plastic drapes sourced from the [redacted], and gloves are sterilised with 100% ethanol. Hair is removed from the skin with a combination of clippers and hair removal cream. The skin is prepped by washing the incision area from inside to out with betadine and then 100% ethanol. A clean gown, hair net and facemask are worn. The biopsy guns are surgical grade and are also sterilized with 100% ethanol between uses. The biopsy guns are typically reused 5 times before being discarded. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. A small incision is made in the abdominal wall and the ovary and oviduct removed before closure with sterile sutures. The abdominal wall and skin are closed separately. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to stauch any bleeding and the skin sutured as required. On the following day, treatment with [redacted] will be commenced. [redacted]) will be administered by subcutaneous injection daily for 25 days, which is the standard procedure that we have used in our previous studies. Mice will be checked and weighed daily until the conclusion of the experiment to monitor their health. Our previous experience with this model demonstrates that the daily injections are well tolerated by the 129/SvEv wildtype mice, which are very amenable to handling.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery. Mice in the [redacted] receive [redacted] on the day of the operation and each day thereafter. After 3-4 days they will be replaced into a communal cage of 2-5 animals.

Mice will recieve [redacted] on days 3, 5, and 7 post-surgery. For [redacted] will be injected into the tail vein on day 4 post-surgery. To [redacted] At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out.

ii) How will the level of anaesthesia be monitored.
Pedal withdrawl (toe-pinch) reflex method of choice for most species
iii) Please describe the frequency and duration of post-operative monitoring

The University of Otago expects that all animals shall be monitored continuously post-anaesthetic until they are fully ambulatory, then twice daily follwoing major survival surgery and once daily following minor survival surgery, for a minimum of 4 days. Standardised monitoring sheets are available.

Alternative monitoring arrangements may be needed when animals are released post-operatively in wildlife studies.

Frequency = number of times per day, Duration = number of days.

Answer

Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

iv) What monitoring sheets will be used?
University of Otago Animal Welfare Monitoring Sheets
v) What are the parameters to be monitored?
Body weight

Food/water intake
Level of activity
Diarrhea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Coat rough/fur on end
Hunched posture
Pain behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear Position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**

Other

Tumour size should not exceed 200 mm^2

vi) Will individual animals undergo multiple surgeries?
Yes

The first procedure is to implant the [redacted] into the fat pad. The second procedure is a biopsy of the tumour before treatment begins. It is vital to have a baseline measurement of the tumour before treatment begins so that we can measure the effect the drugs have on the tumour. An ovariectomy is also performed at the same time, and again this is vital to effectively halt production of oestrogen in the body.

vii) Will paralytic agents be administered in this study?
No
viii) Please confirm that post-operative analgesia will be routinely administered for all but minor surgeries.
Yes, analgesics will be used and details are provided in the RVM table.

5.2.10 Potential Stressors

Describe the potential stressors that animals may experience during procedures conducted on this protocol.
Answer

Isolation stress: Following the initial biopsy and ovariectomy, mice will be housed individually for 3-4 days until the wound has essentially healed and any pain has alleviated. Mice are housed within sight and sound of other mice, and returned to communal housing as soon as possible

Animal number calculation for study segment 5. [redacted]

Mouse		
	Max	Factor
	12	
	12	**Mouse**

7 Manipulations & Procedures

Not applicable

7.1 Description of manipulations & procedures

6. [redacted]

Species to be used for the study segment

Mouse

5.2.1 Study Segment Summary

Briefly explain the experimental design (is this a pilot or main study) and specify all animal manipulations used in this study segment.

*Specific manipulations to be employed in the study must be described on the "Manipulations" tab. The Study Segment Summary should be written in an **animal-centric** fashion to allow the AEC to understand the **experimental course of an animal from its entry into the experiment to the endpoint** of the study. A flowchart of the individual manipulations may be an effective presentation of the study segment. You can attach a flow chart using the attachments button at the bottom of the form.*

Answer

The following procedures will be used for the main study. To [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, dormitor and atropine) and a [redacted] will be injected subcutaneously into the mammary tissue. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring. Mice will be monitored at least twice weekly until tumours become palpable. Once palpable, tumours will be measured with calipers 2-3 times per week until the tumour reaches 49 mm^2. It usually takes about 20-40 days for the tumours to reach 49 mm^2

When a tumour reaches 49 mm^2, a 18-gauge core biopsy will be taken. Mice receive antibiotic (Amphoprim) prior to surgery and will be anaesthetised (Ketamine, Domitor, Atropine). An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive anaesthetic. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to staunch any bleeding and the skin sutured as required.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery, and mice will receive analgesics (Temgesic) twice daily for 3-4 days post-surgery. Mice in the [redacted] receive a [redacted] on the day of the operation and each day thereafter until day 25. After 3-4 days they will be replaced into a communal cage of 2-5 animals. Mice in the [redacted] will receive a [redacted] on the day of the operation and each day thereafter until day 25. Mice in the [redacted] will receive a [redacted]) on days 3, 5, and 7 post-surgery for a total of [redacted] per mouse. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. For mice in the [redacted] will be injected into the tail vein on day 4 post-surgery. At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out. At the end of 25 days, mice will be euthanised and a blood sample will be taken. We will collect the tumour, spleen and lymphoid tissues. The tissues obtained from these experiments will then be analysed to address the experimental objectives. Please see the attached flow chart illustration for further clarification.

5.2.2 Study Segment

Is this study segment the same as any other study segment?
Yes

If the same manipulations are used in each segment, with the same species and the same adverse effects are anticipated, then this section only needs to be completed once.

Please advise which other study segments are the same as this one.

Segments 1-6

5.2.3 Adverse Effects

A) Please indicate if any of the following adverse effects may potentially occur during the proposed study.
Weight Loss
Haemorrhage
Infection
Pain
Other

Tumour ulceration

B) If any of the above adverse effects were selected then please specify how the adverse effect will be managed.

If you have not ticked any of the above adverse effects, please enter n/a

Answer

Adverse side effects, apart from ulceration of the tumour as discussed previously, are unlikely but if a mouse meets any of the standard international humane endpoints we will euthanize the animal immediately. Pain will be well managed with drugs following the biopsy of the tumour and ovariectomy. We have not experienced haemorrhage or wound infection following ovariectomy, but are a risk in all surgeries. Careful surgical technique and administration of antibiotics reduce this to a very low risk. Some weight loss may occur as the tumour increases in size and this will be monitored by regular weighing and assessment of the overall condition of the mouse, however this has not been noticed in any mice as of yet. With any application of anaesthesia to mice, there is likely to be some small amount of weight loss, as the activity and appetite of the mouse is reduced for several hours. However, we would expect less than 10% body weight loss, and the mice will be monitored with twice daily weight measurements for 4 days following surgery. Mice quickly recover the weight loss within a few days, and in the event weight loss is greater than the humane endpoints, the mouse will be euthanized.

C) Detail the survival rates and incidence of adverse events for the proposed manipulations based on the relevant literature and your experience of the manipulations
Answer

This [redacted] has been developed within the last 5 years and, to date, only appears in limited number of papers, which unfortunately do not investigate long term survival rates. Neither paper mention ulcers or adverse effects, however personal communication with one of the authors confirms that ulceration occurred in some cases. We have a successful tumour take rate of 94%, which is similar to other mammary xenografts.

Our efforts to refine the model including reducing the [redacted] injected have reduced the rate of ulceration amongst our mice to below 20% (down from ~25%). Some ulcers formed quickly before the tumour was large enough to begin treatment, and others developed after the initial biopsy at any time ranging from 4 - 23 days post biopsy and commencement of treatment. Ulcers may occur for a variety of reasons, and we have addressed each of these causes in the attempt to reduce ulceration. We have confirmed the [redacted] are free from bacteria and mycoplasma, and that the [redacted] are being injected into the mammary gland, not subcutaneously. We are using the smallest volume of injection possible under sterile conditions and prepare the tumour carefully for biopsy. We have explored the possibility of using alternative, more invasive, surgical techniques to [redacted] with the aid and supervision of the [redacted] in 2014 and in November 2017 but, to date these pilot animals showed that [redacted] into the mammary fat pad was more effective using our current method. We also discussed alternative approaches with [redacted] and [redacted] recommended this method was most effective, based on the large number of animals [redacted] had used mammary fat pad implantation on. We have also discussed a change to softer bedding with [redacted]. It is known that some [redacted] are more prone to ulceration than others, and unfortunately it appears [redacted] are one of these, however there are no other suitable [redacted] that we can utilise for this study.

Ovariectomy is a routine procedure carried out in many mouse facilities around the world, and [redacted] has had very high survival rates following ovariectomy in our previous study. The tumour biopsy results in minor bleeding which is easily controlled and due to the small gauge of the needle there is only a small wound in the skin which heals well. Mice will be treated with antibiotics and painkillers and we expect minimal complications following surgery. Ovariectomy can cause adverse effects over a longer time period, however given the short length of the study, we do not expect any of these to occur.

[redacted] works by lowering the production of [redacted], and is unlikely to have any significant effects on the mice. Some papers have reported lower uterine weights and some changes in bone density, but as mice are only exposed to [redacted] for 25 days, we have not observed any significant effects on the mice. We have investigated the possibility of an [redacted] to deliver [redacted]. However, the pumps available are only able to carry a sufficient volume of [redacted] to last 50 hours so would have to be refilled every second day which we believe negates the advantage of using the pump. In addition, the mice must be handled every day regardless of whether we use [redacted]. Currently, we are able to combine the weighing, monitoring, and injection into one event. Moreover, the concentration of [redacted] would need to be increased. Previously when using a higher concentration of [redacted], we observed a local skin reaction, which we were able to eliminate by reducing the concentration and injecting a larger volume. We are concerned that with a slow release of a small volume of concentrated drug in a fixed position, that there would be a local inflammatory reaction. The subcutaneous injections allow us to rotate the position of injection to different parts of the body to avoid repeatedly irritating the same site.

Our [redacted] will be prepared according the method developed by our collaborator at the [redacted] which has previously been shown to be well tolerated by mice. In our recent study [redacted] we have encountered some problems with lack of recovery after surgery which is probably due to the timing of the [redacted] and we have changed the timing of the [redacted] to prevent this from happening in the future.

5.2.4 Animal Monitoring

A) Please describe the frequency and duration of animal monitoring over the course of the study

Frequency = number of times per day, Duration = number of days

Answer
Prior to [redacted], mice will be checked a minimum of twice weekly. Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

B) Please attach an example of the monitoring sheet to be used. Please note all sheets (including electronic records) need to be available for review by the AWO upon request.
Other (Please attach a sample to this protocol by using the attachments button at the top of the form.)

C) What are the parameters to be monitored?
Body weight
Food/water intake
Level of activity
Diarrhoea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Body Condition
Coat rough, fur on end
Hunched Posture
Pain Behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**
- **Cheek bulge**

Other
Tumour size should not exceed 200 mm^2

5.2.5 Endpoints

A) Is death an endpoint?

Death as an endpoint, which in biomedical science terms means the animal is left to die as part of the experimental protocol without any alleviation of suffering or human intervention is generally ethically unacceptable and must be fully justified.

No – animals will be killed

B) Indicate any additional humane endpoint criteria (other than those listed above) that may apply to this study.
Please include any other information you can provide on euthanasia endpoints for these animals
Tumour ulceration

C) Experimental Endpoint: What will determine the experimental endpoint(s) of the study?

For example: state the specific time points, specific tumour size, etc.

Answer
Mice will be euthanized at the end of the 25 day drug treatment, or when the tumour reaches a maximum size of 200mm2, whichever comes first.

5.2.6 Euthanasia

A) Describe the method of killing and euthanasia that will be used

Note that some methods may require scientific justification

CO2 chamber

B) Please select the method of ensuring death of the animal
Cervical Dislocation

5.2.7 Breeding

Are you breeding animals with a potential adverse phenotype under this protocol?
No

5.2.8 Animal Identification Methods

Describe method and age at which animals are identified.
Answer

Each animal will be identified with a unique cage card number. In addition, each animal inside the cage will have an ear punch unique to that cage number which allows us to identify individual mice by a unique ID number assigned to that mouse by the research group. Each cage is allowed to house a total of 5 mice, thus, the ear punches will be as follows: 1.) No Mark (NM) 2.) Left Ear (LE), 3.) Right Ear (RE), 4.) Left Ear/Right Ear (LE/RE), and Left Ear x2 (LEx2). Our system allows for the least amount of ear punches possible while still maintaining uniqueness among 5 mice. This will help reduce pain to the mice by using less ear punches. For example, if only 3 mice are in a cage the ear marks chosen will ALWAYS be 1.) NM, 2.) LE, and 3.) RE, resulting in a total of 2 ear punches total; as oppose to using 1.) LE, 2.) LE/RE, and 3.) LEx2, resulting in 5 ear punches total. We have previously tried using ear tags, but noticed that some of the mice developed a red, swollen, inflammed area at the site of the ear tag. Our study is investigating immunological responses in the animal, so the ear tags are not ideal for our studies as they may add in confounding factors regarding the immune response in the mouse. The animals will be identified at the time of subcutaneous [redacted].

5.2.9 Surgery

A) Will you be performing surgery on this study segment?
Yes

i) Will the surgery be survival or non-survival?
Survival surgery

Please describe the surgery to be performed

Please provide the following details on the surgery:

Details of the sterilisation of all equipment, implants, surgical drapes etc and of any re-sterilisation during surgery (between animals).

Details of PPE used and how it is sterilised.

Preparation of surgical site.

Full details of the surgery – including drugs used.

Details of type of ear bars used for stereotaxic surgery.

Details of implants (size and weight).

1st surgery: Tumour implantation

To [redacted], adult wild type female 129S6/SvEv mice will be anaesthetised (ketamine, domitor and atropine) and a [redacted] injected percutaenously into the mammary tissue. The hair is removed by clippers and hair removal cream to assist in locating the nipple. This is a very short procedure and the anaesthesia is required to immobilise the mouse to ensure correct placement of the needle. The area is sterilized with 100% ethanol before the cells are injected. Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered) and then will be replaced into a communal cage of 3-5 animals, with appropriate post-operative monitoring.

2nd surgery: Ovariectomy, biopsy and [redacted]

Mice will be anaesthetised for this procedure and at the same time, they will be ovariectomised, or receive a sham overiectomy (for those mice not receiving [redacted], and have have their tumours biopsied. This is required to prevent any [redacted] production and ensure the success of the [redacted] reatment. Mice receive analgesics (Temgesic) and antibiotics (Amphoprim) prior to surgery. An ovariectomy (or sham) is performed and lopaine is used as a local pre-emptive analgesic. Surgical instruments are autoclaved for each animal, or sterilised between animals using the Germinator 500 bead steriliser in [redacted]. The drapes are single use, sterile, clear, plastic drapes sourced from the [redacted], and gloves are sterilised with 100% ethanol. Hair is removed from the skin with a combination of clippers and hair removal cream. The skin is prepped by washing the incision area from inside to out with betadine and then 100% ethanol. A clean gown, hair net and facemask are worn. The biopsy guns are surgical grade and are also sterilized with 100% ethanol between uses. The biopsy guns are typically reused 5 times before being discarded. Using aseptic techniques, surgery will be carried out by two separate flank incisions of approximately 5mm. A small incision is made in the abdominal wall and the ovary and oviduct removed before closure with sterile sutures. The abdominal wall and skin are closed separately. For the biopsy, the hair on and surrounding the tumour is removed and cleaned with 100% ethanol, then a surgical quality biopsy gun is used to take the sample. Pressure is applied to stauch any bleeding and the skin sutured as required. On the following day, treatment with [redacted] or vehicle will be commenced. [redacted] will be administered by subcutaneous injection daily for 25 days, which is the standard procedure that we have used in our previous studies. Mice will be checked and weighed daily until the conclusion of the experiment to monitor their health. Our previous experience with this model demonstrates that the daily injections are well tolerated by the 129/SvEv wildtype mice, which are very amenable to handling.

Mice will be placed into an individual cage and kept warm until they have recovered from the anaesthetic (antisedan is administered to assist this) with appropriate post-operative monitoring and analgesics (Temgesic). A bolus of 0.5ml saline is given subcutaneously to assist hydration and recovery. An additional dose of Amphoprim is given following surgery. Mice in the [redacted] receive [redacted] on the day of the operation and each day thereafter. After 3-4 days they will be replaced into a communal cage of 2-5 animals.

Mice will recieve [redacted] on days 3, 5, and 7 post-surgery. For [redacted] will be injected into the tail vein on day 4 post-surgery. To [redacted] At the end of the 25 days, mice are euthanized by cervical dislocation and the tumour is dissected out.

ii) How will the level of anaesthesia be monitored.
Pedal withdrawl (toe-pinch) reflex method of choice for most species
iii) Please describe the frequency and duration of post-operative monitoring

The University of Otago expects that all animals shall be monitored continuously post-anaesthetic until they are fully ambulatory, then twice daily follwoing major survival surgery and once daily following minor survival surgery, for a minimum of 4 days. Standardised monitoring sheets are available.

Alternative monitoring arrangements may be needed when animals are released post-operatively in wildlife studies.

Frequency = number of times per day, Duration = number of days.

Answer

Mice will be checked once a day for a minimum of 4 days following percutaneous injection. Mice will be checked at a minimum of twice weekly thereafter, until the tumour reaches a diameter of 7mm (49mm2), at which point we will take the biopsy. Following biopsy and ovariectomy, mice are monitored twice daily for at least 4 days until we are satisfied the mouse has recovered, and from then on inspected daily at the time of drug administration until the end of the experiment (25 days post biopsy in total).

iv) What monitoring sheets will be used?
University of Otago Animal Welfare Monitoring Sheets
v) What are the parameters to be monitored?
Body weight

Food/water intake
Level of activity
Diarrhea
Dehydration (skin turgor test)
Bleeding
Wound condition
Sutures/clips condition
Coat rough/fur on end
Hunched posture
Pain behaviour
- **Writhing**
- **Back arch**
- **Stagger**
- **Belly Press**
- **Falling over**
- **Twitch/tremor**

Mouse Grimace Scale
- **Ear Position**
- **Eye squeeze**
- **Whisker change**
- **Nose bulge**

Other

Tumour size should not exceed 200 mm^2

vi) Will individual animals undergo multiple surgeries?
Yes

The first procedure is to [redacted] into the fat pad. The second procedure is a biopsy of the tumour before treatment begins. It is vital to have a baseline measurement of the tumour before treatment begins so that we can measure the effect the drugs have on the tumour. An ovariectomy is also performed at the same time, and again this is vital to effectively halt production of oestrogen in the body.

vii) Will paralytic agents be administered in this study?
No
viii) Please confirm that post-operative analgesia will be routinely administered for all but minor surgeries.
Yes, analgesics will be used and details are provided in the RVM table.

5.2.10 Potential Stressors

Describe the potential stressors that animals may experience during procedures conducted on this protocol.
Answer

Isolation stress: Following the initial biopsy and ovariectomy, mice will be housed individually for 3-4 days until the wound has essentially healed and any pain has alleviated. Mice are housed within sight and sound of other mice, and returned to communal housing as soon as possible

Animal number calculation for study segment 6. [redacted]

Mouse		
	Max	Factor
	12	
	12	**Mouse**

7 Manipulations & Procedures

Not applicable

7.1 Description of manipulations & procedures

Total number of animals

Species	Max
Mouse	72

6.3 Animal Numbers Justification

6.3.1 Is this a quantitative or non-quantitative study?

Include how the minimum and maximum numbers were determined and what criteria were used to determine group size. Be sure to include any literature references of statistical analysis performed (ex. power analysis) as well as provide any previous experience from similar studies. You can attach references using the attachments button at the bottom of this page, or include hyperlinks within your justification

quantitative study

Each group will have 10 mice which has been powered to detect a standardised difference of 0.42 or greater in [redacted]. Each group will have 10 mice which has been powered to detect a standardised difference of 0.42 or greater in [redacted] n the paired pre- and post treatment samples (90% statistical power). The tissues obtained from these experiments will be analysed by immunohistochemistry and a subset will be analysis using flow cytometry analysis. The primary endpoint of this phase of the study will be a difference in tumour area. Ten mice per group will give 90% power to detect a standardised difference of 0.23 or greater. We have allowed an additional two extra mice per group in the event some of the tumours ulcerate before the treatment is complete.

6.3.2 Have you had a statistician review the animal numbers?

While a statistician review and signature are not standard requirements, they are strongly encouraged, particularly when data from different groups are being compared.

Yes

Please provide name of statistician and attach report if provided

You can either attach the statisticians report or add them as a signer on the personnel tab.

We are currently consulting with the [redacted] to meet with a statistician to perform a power analysis for the current animal study and our future animal study [redacted]. However, our previous studies based on power calculations performed by a [redacted], have achieved significant differences approximately 10 animals per group. This suggests that our original power calculations were reasonable.

8. Drugs/Other Agents

8.1 Restricted Veterinary Medicines (RVM)

Procedure	Species/Strain	Drug/Agent	Route	Dose rate	Frequency (# per day)	Duration (# of days)
Survival RVM Procedure	Mouse	Ketamine injection	SC	75 mg/kg	1	1
Survival RVM Procedure	Mouse	Buprenorphine / Temgesic	SC	0.05 mg/kg	2	4
Survival RVM Procedure	Mouse	Medetomidine / Domitor	SC	1 mg/kg	1	1
Survival RVM Procedure	Mouse	Atropine	SC	0.05 mg/kg	1	1
Survival RVM Procedure	Mouse	Trimethoprim & Sulphonamide / Amphoprim	SC	30 mg/kg	BID	1
Survival RVM Procedure	Mouse	Atipamazole / Antisedan	SC	5 mg/kg	1	1
Survival RVM Procedure	Mouse	Lidocaine/Lignocaine/Lopaine /Xylocaine	SC	<4 mg/kg	1	1

Additional RVM Information

Are RVM used in this study?

B) Where will the RVM be stored?

Own Laboratory

Please provide details of your research laboratory 'Drug Control' contact person - name, department, telephone (work & emergency), and email.

Answer

[redacted]

Please provide details of your drug safe. (Safe number, building, floor, room, drug register number)

Answer

[redacted]

C) Please confirm your responsibility to supervise personnel administering Restricted Veterinary Medicines (RVM)

Note for Principal Investigator: If you are not administering RVM personally, this does not negate your obligation to ensure the competency of administration of RVM by members of your research group.

I acknowledge responsibility of administration of RVM to animals by members of my research group and will ensure they have obtained appropriate supervision, training and level of competency before allowing their administration of RVM

Please list all personnel who will be handling (including collecting) or administering RVM.

[redacted]

8.2 Other Agents

Procedure	Species/Strain	Drug/Agent	Route	Dose rate	Frequency (# per day)	Duration (# of days)
Tumour Biopsy, [redacted], and Ovariectomy	Mouse					

Additional Other Agent Information

Please provide the volume and concentration for each experimental agent being administered.

Answer

Species/Strain	Drug/Agent	Route	Dose Rate	Frequency (#/day)	Duration (# of days)	Volume
Mouse	[redacted]	SC	1 mg/kg	1	25	0.18 ml
Mouse	[redacted]	IV	[redacted]	1	1	0.20 ml

Mouse	[redacted]	IP	250 ug	1	3	0.08 ml
Mouse	[redacted]	IP	250 ug	1	3	0.08 ml
Mouse	[redacted]	IP	250 ug/100 ug	1	3	0.12 ml

Will you be using other non-RVM agents in this study?

9. Personnel Contribution & Training

9.1 Principal Investigator Qualifications

Describe the Principal Investigator's Qualifications to perform or oversee the research.
Answer

[redacted] has completed "Module 1: Ethics and Legislation" and "Module 2: Experimental Techniques" of the AWO Training Program. [redacted] has successfully overseen 4 AUP approved protocols including [redacted] in the past, and [redacted]. [redacted] also observes the work undertaken by [redacted] at regular intervals.

9.2 Required General Training

CS7 Euthanasia
Intro to Restricted Veterinary Medicine
Module 1 Ethics and Legislation
Module 1 Practical - Mouse
Module 2 Practical - Mouse
Module 3 Anaesthesia and Surgery
Suturing Wetlab

9.3 Required General AUP Training for Study Personnel

	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
CS7 Euthanasia	NO	NO	NO	YES	NO
Intro to Restricted Veterinary Medicine	NO	NO	YES	YES	NO
Module 1 Ethics and Legislation	YES	YES	YES	YES	NO
Module 1 Practical - Mouse	NO	YES	NO	NO	NO
Module 2 Practical - Mouse	YES	NO	NO	YES	NO
Module 3 Anaesthesia and Surgery	NO	NO	YES	YES	NO
Suturing Wetlab	NO	NO	NO	NO	NO

	[redacted]	[redacted]	[redacted]
CS7 Euthanasia	NO	YES	NO
Intro to Restricted Veterinary Medicine	YES	YES	YES
Module 1 Ethics and Legislation	YES	YES	YES
Module 1 Practical - Mouse	YES	YES	YES
Module 2 Practical - Mouse	YES	YES	YES
Module 3 Anaesthesia and Surgery	YES	YES	YES
Suturing Wetlab	NO	YES	NO

9.4 Personnel Contribution and Qualifications

A) List the contribution and qualifications of all personnel.

Please specify:

- *which procedures and/or monitoring (including euthanasia) are performed by individual personnel. When there is surgery, please state whether it is survival or non-survival.*
- *What qualifies the individual to perform the procedure - include experience and specific training (other than module training). A list of degrees awarded may be relevant but is NOT sufficient.*
- *When a student is involved - please state the course and year of study.*

Answer

[redacted]

B) Please confirm the appropriate experience of personnel to perform all manipulations

Please note that the completion of Animal Welfare Office module training alone is not sufficient to deem personnel fully competent.

I confirm that all personnel that are required to perform manipulations are fully competent

10. Animal Husbandry/Housing

10 Animal Husbandry & Housing

Is this section relevant to your study?
Yes

10.1 Dietary Manipulation

Will there be any dietary manipulation on this protocol?
Yes

Mice will be fed a soya free cereal grain based diet (AIN93) devoid of phytoestrogens from Specialty Feeds, Australia. Phytoestrogens have been shown to bind to some extent to [redacted] and can affect tumour growth, so this diet will avoid any of these potential complications.

10.2 Deviations from Standard Environmental Enrichment

Will there be deviations from standard environmental enrichment on this protocol?
No

10.3 Husbandry and Other Considerations

A) Will there be other husbandry deviations or special considerations regarding the animal use in this protocol?

For example, metabolic cages, non-IVC cages, biobubble housing, specialised diets and/or water, reverse light/dark cycles, individual housing, specialised waste disposal, behavioural study equipment etc.

Yes

Mice must be kept in specific pathogen free rooms. The mice must be kept in rooms that have been deemed as virus free on the [redacted].

B) Provide details of any manipulations that may cause animals to deviate from standard behaviour - particularly those that it will be important for the animal technicians to know. Include the nature of the change and for how long it will occur.

These may include such things as changes in gait, behaviour, abnormal secretions. Recovery from anaesthetic or obvious surgical interventions need not be listed. If technicians observe the listed changes they will not intervene unless there appears to be a welfare issue at which stage they will follow standard sick reporting protocols.

Location Assignments

Site	Building	Floor	Section	Room	Room Type
[redacted]	[redacted]	[redacted]		[redacted]	[redacted]

10.4 Location Assignments

A) Please provide information on which procedures you will perform in each room.

Please include duration of time the animal will be kept in the procedure room (or be away from their usual housing).

Answer

[redacted] - animals will be housed in this room in IVC cages. All experimental procedures including survival surgery will be performed in this room in the biological safety cabinet hood.

11. Safety & Compliance

11.1 Use of Uncleared Biologicals and Cell Lines

Are human or animal derived Biologicals or Cell Lines used on animals on this protocol?
Yes

Please add the appropriate safety/compliance officer as a signer on the Personnel tab.

Do you have the appropriate approvals in place?
Yes

[redacted]

11.2 Use of Infectious Agents

Are Infectious Agents used on animals under this protocol and listed above?
No

11.3 Use of rDNA or RNA e.g. CRISPR-CAS, zinc finger, RNAi

Are Genetic Constructs (rDNA or RNAi) used on animals under this protocol?
No

11.4 Use of New/Genetically Modified/Unwanted Animals

Will New/Genetically Modified/Unwanted Animals be used on this protocol?
No

11.5 Use of Chemical Hazards

As part of this protocol, are any substances (not including anaesthetics, analgesics or veterinary medicines) to be administered to animals that pose a potential health hazard to animal users or animal care technicians?
Yes

Do you have SOPs and/or a Safety Plan in place?
Yes

11.6 Use of Radiation Substances

Are you using radioisotopes or xrays/radiation equipment in this protocol?
No

11.7 Waste and Animal Disposal Procedures

A) Describe the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study.
Answer

For hazard identification there are signs on animal room doors and animal cages when hazards are used in animals maintained after hazard administration. For safe handling personal protective equipment will be used including disposable gloves, gown (lab coat), face mask, hair net, and shoe covers to prevent cross contamination from outside the room to inside and vice versa. For disposal of contaminated bedding, cages, equipment or dead animals we will use the institutional waste disposal system.

B) Describe methods for removal of radioactive, chemical or infectious waste and, if applicable, the monitoring of radioactivity.

Please enter n/a if not applicable to this protocol

Answer

N/A

11.8 Additional Safety Considerations

Describe any additional safety considerations that apply to this protocol which are not already listed above.

12. Transportation

12 Transportation

Will animals be transported temporarily or permanently to a satellite facility or laboratory?

Translocation of animals in the wild is addressed in the Field Studies tab.

No

13. Field Studies

13 Field Studies

A) Will there be field studies associated with this protocol?
No

B) Will any of the animals used in this study enter the human or animal food chains?
No

C) Will any animal be treated with any medicine or other agent be released to the wild?
No

14. Teaching Protocol

14 Teaching Protocol

A) Is this a Teaching Protocol or does it have a major teaching component?
No

15. PI Declaration

PI Certification

I confirm that I have discussed Safety & Compliance hazards, restricted veterinary medicines and controlled medicines with appropriate compliance team members and added tham as signers where applicable.
Yes
[redacted]

I hereby certify and undertake that:

1. I will comply with the conditions for the use of animals in research, testing and teaching as defined in the New Zealand Animal Welfare Act 1999 and the Animal Welfare Act Amendment 2015 and the controls on Restricted Veterinary Medicines as defined in the Agricultural Compounds & Veterinary Medicines Act 1997.

2. Animals to be used will be lawfully acquired, treated with care, adequately fed and watered and accommodation will be adequate in size and will be maintained in a hygienic condition.

3. During all but the most minor procedures, appropriate anaesthesia and analgesia will be given to eliminate sensitivity to pain. Post-operative care will be such as to minimise discomfort and pain and will accord with standard laboratory animal veterinary practice.

4. Experiments are designed to avoid unnecessary sacrifice of animals and care has been taken to select the optimum species for a given experiment and to utilise the optimum methodology.

5. The staff member responsible for an experiment will ensure that persons performing any procedure for which s/he holds ethical approval possesses the necessary expertise, or will ensure that the experimenter is supervised by a person possessing such expertise and that procedures are carried out in the manner stipulated in this application and that only procedures listed in this protocol will be used.

6. Any later modification to the procedures approved (e.g. techniques, animal numbers, personnel, etc.) will be submitted to the Animal Ethics Committee for approval. I accept responsibility to ensure that all personnel listed on the application and the Head of Department are made aware of changes to applications; both modifications to existing applications and any proviso conditions specified during the approval process.

7. I agree to comply with the AEC site visit programme and provide a protocol evaluation report at the end of the study.

8. I have read the University of Otago's Code of Ethical Conduct for the Manipulation of Animals and agree to abide by the conditions therein. Failure to comply will result in withdrawal of my approval to use animals.

9. The other personnel named in the application have read, and are familiar with, the contents of this application.

10. I will comply with the university policies for the purchase and management of Restricted Veterinary Medicines.

11. I confirm that I will use only the Restricted Veterinary Medicines listed in this Animal Ethics Committee protocol.

I certify that the above statements are understood and will be followed.

[redacted]

Because we are not sure if [redacted]

[redacted]

Protocol Closure

Protocol Closure

[redacted]