



Home Office

## NON-TECHNICAL SUMMARY

# Development of an implantable device for the treatment of brain tumours

### Project duration

5 years 0 months

### Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

### Key words

Brain, Cancer, Technology, Therapy

### Animal types

Mice

### Life stages

adult

## Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

## Objectives and benefits

**Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.**

**What's the aim of this project?**

To develop and test an implantable device, which has been designed to deliver chemotherapeutic drugs for the treatment of brain tumours.

**Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.**

**Why is it important to undertake this work?**

With more than 11,000 new cases each year in the UK, brain tumours are a common disease which significantly impacts the life of affected patients. On average, only 12% of patients survive for five or more years after the diagnosis.

Depending on each individual case, brain tumours are treated by a combination of chemotherapy, radiotherapy and, if possible, surgical removal. A fundamental limitation for the success of chemotherapy is the blood-brain-barrier, a membrane that separates the sensitive brain tissue from the blood and any toxic substances that may be dissolved in it. It plays a pivotal role in protecting the brain from toxic substances but also significantly reduces the amount of cancer drugs which can be delivered into a brain tumour through the blood. Additionally, reduced blood flow to solid brain tumours further complicates the process of delivering enough drug to have a positive effect. The highly toxic nature of chemotherapeutic drugs limits the volumes that can be safely given to a patient because they can cause damage in healthy tissues as well as the tumour being targeted.

A different approach for enhancing transport through the barrier is avoiding it entirely by delivering cancer drugs directly into tumours. Convection enhanced delivery, for instance, is a technique where a thin tube is inserted into a brain tumour through which a cancer drug solution is injected. By creating a constant overpressure of the solution, the drug is forced into the tumour which allows better drug distribution than simply soaking the tumour in drug solution. However, convection enhanced delivery is significantly limited by the tendency of the drug to flow back out of the tumour through the hole created to insert the tube. To avoid this problem we will develop a device that can be implanted inside a brain tumour and will deliver anti-cancer drugs without pushing liquid into the brain. The anti-cancer drug, dissolved in fluid, will flow into the implanted device through an input tube. Once inside the device, an electric current will be used to separate the drug from the fluid so that only the 'dry' drug is delivered to the tumour. This is known as iontophoretic drug delivery. The fluid, now cleared of anti-cancer drugs, can be taken away through an output tube. We anticipate that this approach will solve the problems caused by convection enhanced delivery.

Iontophoresis has been an active topic of research for over a hundred years and was traditionally used to increase absorption of a topically applied drug through the skin. This allowed for the safe and painless delivery of drugs, through tissues that are typically impermeable, without the need to puncture the skin with needles and potentially invite infection. Traditional iontophoretic devices consist of a counter electrode and a working electrode, the latter of which is separated from the target tissue by a reservoir

of the chosen drug. When a current is applied across the electrodes the drug is driven out of the reservoir directly into the target tissue. Between 1900 and 2019 almost 2800 publications have been published on iontophoresis and in the year 2020 alone there were 20 papers. The vast majority describe iontophoresis as a method for moving substances through skin but we hope to show that it can be used to deliver drugs within the body, with great accuracy and in a way that doesn't increase local pressure.

### **What outputs do you think you will see at the end of this project?**

The output will be an effective, safe implantable device which will deliver drugs directly into tumours. We will also publish our findings in scientific journals which will include details on our design process and experimental methods.

### **Who or what will benefit from these outputs, and how?**

At the time of writing there is another device in development within our group which aims to utilise the same delivery method for anti-seizure medication into the brain to treat epilepsy. The lessons we learn from the development of the cancer treatment device, from the choice of materials to the implantation method, will feed directly back into the decision making process for manufacturing and testing the epilepsy treatment device and all future devices with similar function. We will also be able to compare the function and efficacy against devices that have been, and are being, developed by other groups around the world.

In the long term, the publications that arise from the data collected during this project are likely to show, for the first time, that drug delivery devices of this type can be used in the treatment of brain tumours. Many anticancer drugs, including cisplatin, cannot penetrate the barrier that separates the brain from the blood and therefore cannot be utilised in this role. If we are able to show that our device can shrink tumour size or reduce toxic damage to non-tumour tissues above and beyond what we would see with the current best available treatment, then we can move forward with the development of a device for human applications. Direct delivery of anticancer agents into brain tumours has the potential to improve the poor survival outcomes associated with brain tumours that are difficult to surgically remove.

### **How will you look to maximise the outputs of this work?**

We will use the data generated, both significant and non-significant, under this project license to inform a number of research papers that we aim to publish in high-impact scientific journals. This should ensure that the outcomes of this work are recognised by a multi-disciplinary audience.

This work is part of an Interdisciplinary Research Collaboration (IRC) involving researchers from five leading UK academic establishments. At every step of the process there is constant communication between the researchers and the clinicians ensuring that our results feed directly back into the hospital setting.

### **Species and numbers of animals expected to be used**

- ♦ Mice: 600

# Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

For this study we will use adult mice with a well characterised background and no deliberate alterations to their genetic makeup. These mice are ideal for our studies because the tumour cells that will be grown under the skin come from mice with a similar genetic background. It is unlikely that the tumour tissue will be rejected by the host so there is no need to use mice with compromised immune systems in order to grow the tumours. The animal facilities at our establishment are very well adapted to caring for small rodents and no major special provisions will need to be made prior to our experiments.

We will use adult mice because the device we are developing will ultimately be used in humans. Testing its function in anything smaller than adult mice will likely prove irrelevant and make the surgical implantations unnecessarily difficult. Whilst it would be beneficial to test the device in a much larger animal (e.g. pig) we believe that the information we get from mice will be sufficiently translatable into the clinical setting.

Typically, what will be done to an animal used in your project?

Diagram showing the 3 scenarios likely to be experienced by the animals.

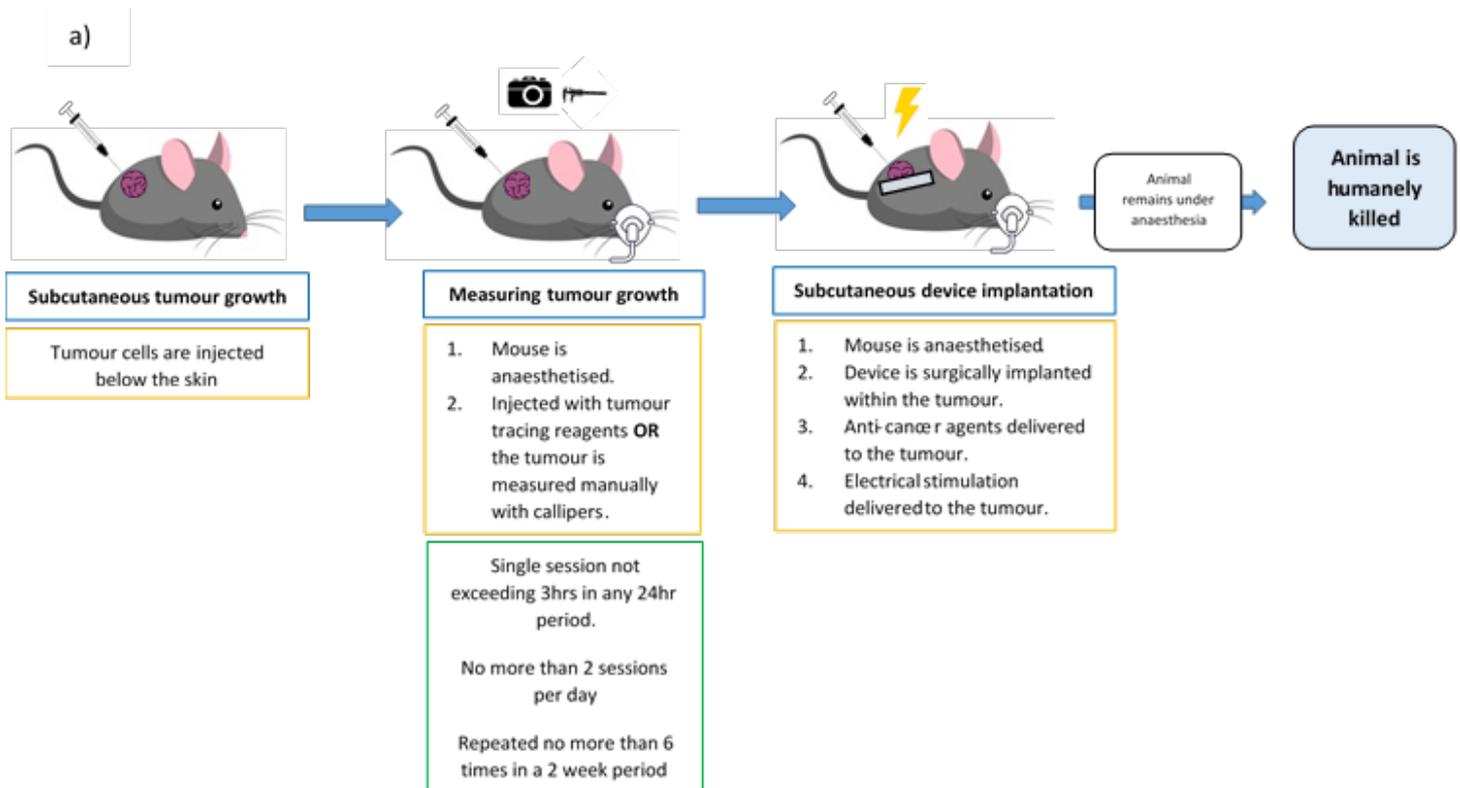


Figure a) Mice will have tumour cells injected subcutaneously which may require general anaesthetic and pain relief. The tumour will be allowed to grow to critical size and measured, using either specialised imaging hardware, for which the mice may be anaesthetised and injected with tumour tracking reagents, or manually with callipers, which may also require general anaesthetic for accurate recording. Once the tumour has reached the correct size the animal will be anaesthetised and the drug delivery device will be implanted within the tumour tissue. Anti-cancer agents and control substances may be applied to the tumour tissue alone or in combination with local electrical stimulation. Before the mice recover from the anaesthesia they will be humanely killed.

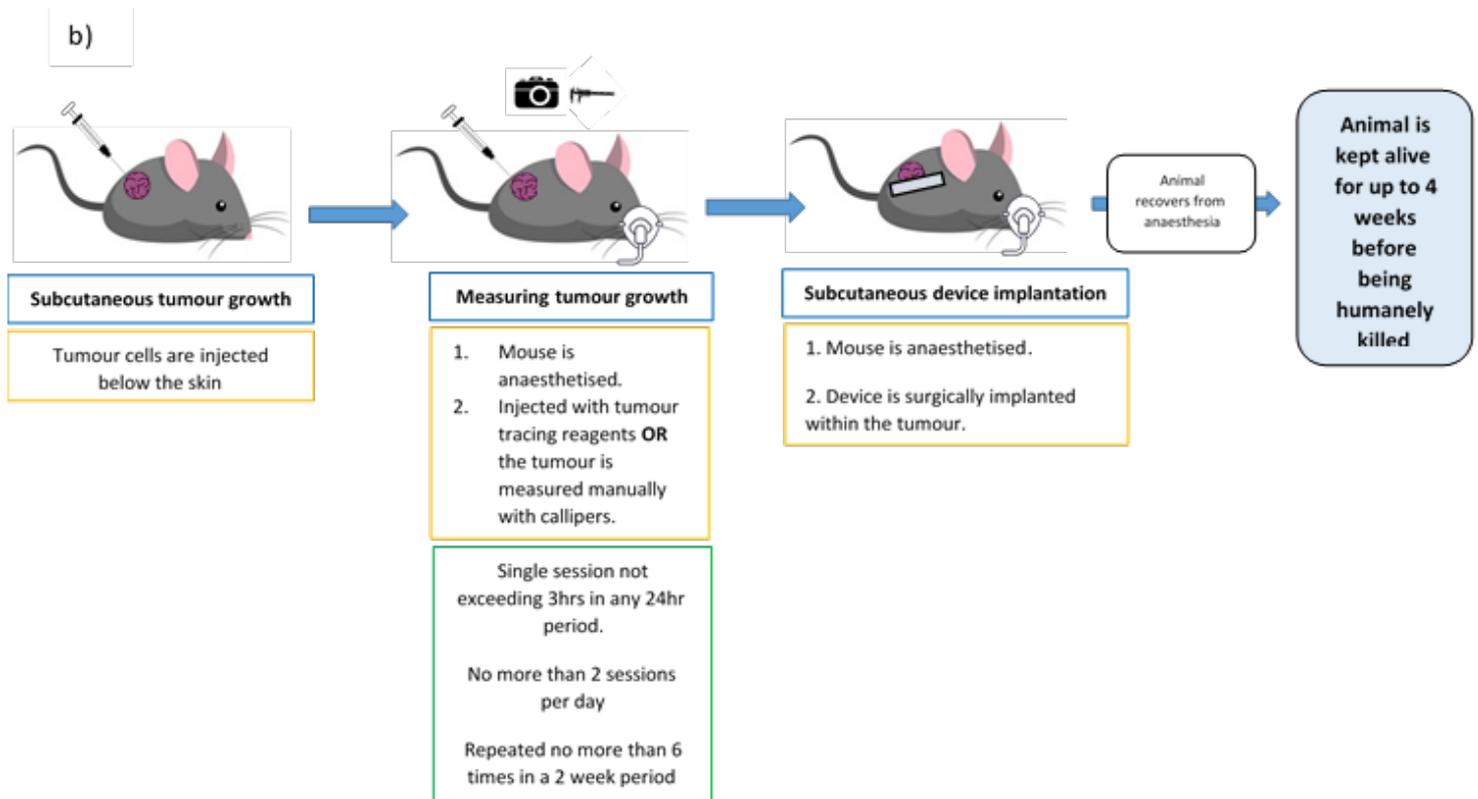


Figure b) Mice will have tumour cells injected subcutaneously which may require general anaesthetic and pain relief. The tumour will be allowed to grow to critical size and measured, using either specialised imaging hardware, for which the mice may be anaesthetised and injected with tumour tracking reagents, or manually with callipers, which may also require general anaesthetic for accurate recording. Once the tumour has reached the correct size the animal will be anaesthetised and the drug delivery device will be implanted within the tumour tissue. The animal will recover and spend up to 4 weeks carrying the inactive device under the skin before being humanely killed. We will analyse the samples from the implantation site to determine how the local tissue environment responds to the device over time.

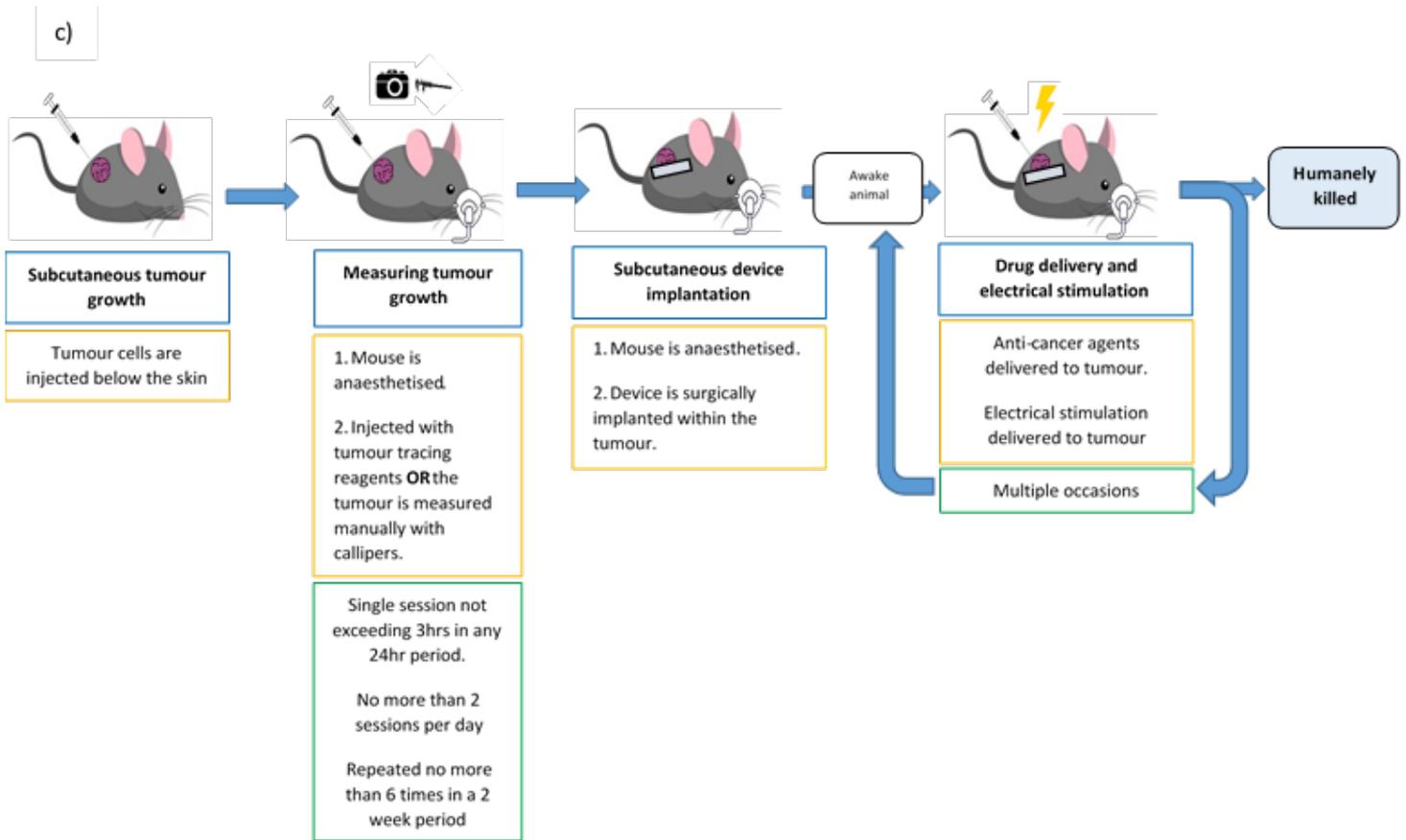


Figure c) Mice will have tumour cells injected subcutaneously which may require general anaesthetic and pain relief. The tumour will be allowed to grow to critical size and measured, using either specialised imaging hardware, for which the mice may be anaesthetised and injected with tumour tracking reagents, or manually with callipers, which may also require general anaesthetic for accurate recording. Once the tumour has reached the correct size the animal will be anaesthetised and the drug delivery device will be implanted within the tumour tissue, again requiring pain relief during recovery. After recovering from anaesthesia, anti-cancer agents and control substances may be applied to the tumour tissue alone or in combination with local electrical stimulation. The device will be operationally tested for a short time (up to one week) or over longer timelines (up to 4 weeks). At the end of the experiment the animal will be humanely killed.

Ideally, we would want to test the device in a brain tumour and brain with similar dimensions to that of a human, better replicating the target tissues and producing more relevant information. However, for the initial validation phases we believe that we can address gain valuable information using brain tumour cells grown under the skin of mice.

### What are the expected impacts and/or adverse effects for the animals during your project?

We expect that the mice may experience mild and transient pain following surgery, however this will be countered using pain killers before and after surgery. Any further surgical complications, although unlikely, will be monitored for and advice will be sought from the Named Veterinary Surgeon should welfare concerns occur.

Humane endpoints refer to one or more physiological or behavioural signs that have been determined to signal when an experimental animal's distress should be terminated or reduced. This is typically achieved by ceasing a painful procedure, administering pain killers or humanely killing the animal.

We don't expect any serious adverse effects to the animal's general well-being during the tumour growth phase as tumour volume measurements will be carried out periodically to ensure that humane endpoints are not exceeded. Most of negative clinical signs associated with tumours in mice present as weight loss and any mice that lose 15% of their pre-implantation weight will be humanely killed.

It is possible that adverse effects will occur in response to administration of the anti-cancer drugs, such as local tissue damage due to direct contact with the agents or an allergic reaction. Adverse effects that are expected include typical signs of toxicity associated with administration of anticancer agents. These may include acute effects such as inactivity or tensing of the abdominal wall to protect inflamed and painful organs. Adverse effects may also include chronic drug effects such as gut toxicity, caused when the toxic drug kills off the friendly bacteria that inhabit the gut and is most likely to manifest as weight loss. Chemotherapy is cleared from the body through the kidneys which can have a damaging effect on them, the resulting water imbalance that often presents as dehydration or, in some case, water retention. The extent of the damage is largely dependent on the individual dose, frequency and cumulative dose of the drug and also manifests as weight loss.

Platinum-based chemotherapeutic drugs such as cisplatin have been linked to permanent hearing loss in human cancer survivors and studies have shown that the same symptoms can effect mice enrolled on cisplatin treatment regimens. While not immediately threatening to the lives of the mice, it may interfere with their normal social behaviour.

### **Expected severity categories and the proportion of animals in each category, per species.**

**What are the expected severities and the proportion of animals in each category (per animal type)?**

Moderate severity, 100% of mice

**What will happen to animals at the end of this project?**

- ♦ Killed

## **Replacement**

**State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.**

**Why do you need to use animals to achieve the aim of your project?**

It is critical that animals are used in the validation of this device because it is not yet possible to simulate the complex tumour environment outside of a living animal. Our aim is to get the device into

the clinical setting where it can help patients with potentially life threatening diseases. Testing the safety of medical devices in animals before they are introduced in human clinical trials is required for both ethical and legal reasons.

### **Which non-animal alternatives did you consider for use in this project?**

Several experiments have been conducted to gather as much preliminary information as possible without the use of live animals.

To measure the speed that the drug moves through the tissue after being released by the device, we cast the device in gel designed to be similar in density and mineral content to living tissue. The drug, bound to a coloured dye, was then released into the gel at varying rates and the speed that the drug travelled through the tissue was measured.

While the gel is a useful model for tissue, we also conducted similar tests using chicken breast and pig brain, purchased from the local butchers to get a better picture of how the drug moves through tissue in relevant biological material.

We have also run simulations, using computer software, that mimic the chemical and physical processes that will occur in the activated device.

### **Why were they not suitable?**

Due to the delicate nature of the brain, the potentially damaging effects of chemotherapeutic drugs and the complex nature of cancer, there are no non-animal alternatives that would provide the peace of mind required to safely move the device into human trials. Non-animal alternatives are suitable for providing preliminary information which allows us to reduce the numbers of animals and refine the experiments but they do not satisfy the need to replace animals entirely.

## **Reduction**

**Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.**

### **How have you estimated the numbers of animals you will use?**

Estimates of animal numbers are derived from a combination of 2 factors: our experience in developing implantable devices - particularly that derived from our previous licence, and our engineering knowledge on the typical number of new versions necessary to develop a successful device. Over the course of this licence, we expect to develop a progressively improved drug delivery device. From our previous experience, we expect to have to go through approximately 20 new versions of devices before arriving at the final design. From our experience, each new version requires approximately 25 animals

to test and obtain decisive feedback on. While difficult to predict the exact numbers at this stage, this calculated estimation provides a good approximation to the number of animals we expect to use.

**What steps did you take during the experimental design phase to reduce the number of animals being used in this project?**

Where possible we intend to randomise the allocation of animals into either control or treatment groups to minimise systemic bias and improving the reliability of the results. We also intend to utilise blinding to ensure that the person analysing the results isn't subject to unconscious bias, further increasing reliability and reducing the requirement for repeated experiments. We will use an experimental design assistant (<https://www.nc3rs.org.uk/experimental-design>) and PREPARE guidelines (<https://norecopa.no/prepare>) to aid in planning the experiments. Our group constantly reviews the work in the field of bioelectronics to avoid unnecessary repetition of animal experiments. We will use the online platform SyRF (<http://syrf.org.uk/>) to aid with our reviews.

**What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?**

Pilot studies will be used to assess feasibility and outcome measures of the experimental plan, mainly regarding new treatments. Previous experience has shown that many potential problems can be identified and rectified in small pilot studies. This means that eventual definitive studies with larger groups of animals have a high probability of success, and large studies will not need to be repeated due to unforeseen study failures.

In order to ensure that the results are reported to the highest standard we will adopt the advice given in the ARRIVE guidelines. By doing so we hope to improve the reproducibility of our animal work.

## Refinement

**Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.**

**Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.**

To test and validate the device in a relevant system we will need to generate a model of glioma. Ideally, we would want to test the device in a brain tumour and brain with similar dimensions to that of a human, better replicating the target tissues and producing more relevant information. However, for the initial validation phases we believe that we can address our objectives using brain tumours grown under the skin of adult mice.

In order to refine our protocols and reduce that chance of causing unnecessary pain to the animals we will work in a step wise fashion, collecting as much information from low risk methods, before

progressing onto methods with a higher risk of complications. We will first test the device in mice that have been terminally anaesthetised, almost completely removing any pain, suffering or distress that could be experienced before the animal is humanely killed. Once we know that we can implant the device safely we will move into tumours that have been implanted under the skin for the next stage of testing. This will allow us to test the ability of the device to deliver drugs to a living tumour and its suitability as a long term wearable device without any risk of neurological complications.

The tumours will be implanted under the skin while the mice are under general anaesthetic with both pre-operative and post-operative pain relief. This ensures that animals don't experience distress but also improves the reproducibility as even small movements during implantation can have negative results on tumour uniformity.

Growing tumours in mice requires close monitoring as the cancers can expand rapidly and spread to other tissues in the body. To counter these risks, we will perform regular volume measurements using non-invasive calliper measurements or bioluminescent imaging. This allows us to take accurate measurements without using procedures anymore invasive than an injection and ensures that we don't allow the tumours to grow beyond the humane endpoints. Measurements will be taken under general anaesthetic to reduce movement and ensure the greatest accuracy.

Mice are a very useful species for cancer research because the disease is so well characterised after decades of research and publications. We know which procedures are likely to cause pain, which signs to look for and how to address any problems. By building on the work of others, we are able to learn from the lessons of the past and improve our methods for the benefit of the animals and our research.

### **Why can't you use animals that are less sentient?**

Adult mice represent the smallest and least sentient animal that we could test our device, intended for human use, while still collecting relevant data. Using younger mice would likely make our surgical interventions more technically difficult than necessary and pose more risk to the animals.

We will use mice that have been placed under terminal anaesthesia for the initial stages of device validation, giving us the opportunity to test the function of the device in living tissues without the risk of complications that may cause suffering. As the device is being developed for chronic human use we will need to move testing into awake animals in order to show that it can be worn for extended periods of time, without malfunctioning or impeding the normal movement of the wearer.

### **How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?**

The mice will be housed in groups, between three and four per cage, to stimulate their normal social behaviours. This is beneficial for the animal's mental welfare and, because distressed animals can become anti-social, allows us to visually assess their general health.

Following surgery the mice will have their surgery sites visually inspected and be weighed at least twice a day for the first 3 days, then once a day for as long as necessary. Weights and observations will be recorded on post-operative data sheets, ensuring a comprehensive account of each mouse's recovery. Mice that show signs of chronic distress such as erect hair, hunched posture, anti-social

behaviour and weight loss of 15% of pre-treatment body weight will be humanely killed. We will also refer to the mouse grimace scale to recognise pain, assess its severity and take steps to relieve it.

Special nesting material and easy-to-eat food will be provided after surgery, as well as heat packs attached to the outside of the cage to aid recovery. Due to their large surface-area to body-volume ratio, small rodents are particularly vulnerable to hypothermia following surgery and the use of heat packs will prevent this.

We will utilise bioluminescent imaging to track the tumour growth over time. This technology requires the tumour cells to be genetically altered so that they carry the gene responsible for emitting light in fireflies. This gene will cause the tumour to glow inside the mouse, producing light that can be detected using a sensitive camera. Computer software will then determine the size of the tumour with greater accuracy than can be achieved using manual methods. The mice will need to be injected with a harmless reagent to stimulate the bioluminescence and briefly placed under anaesthetic for a clear image to develop, but the increased level of accuracy will make sure that the tumours aren't allowed to grow larger than necessary.

We will use non-tail handling methods, such as cupping and tube handling, (<https://www.nc3rs.org.uk/mouse-handling-tutorial>) and enrichment aimed at improving their quality of life. Every animal will be given at least a week to acclimatise to the new facility before any procedure is carried out. All our work is structured in a stepwise approach to only progress with interventions which are more likely to be successful. To achieve this aim, we will carry out initial work in mice under terminal anaesthesia.

### **What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?**

To ensure that we are providing the best and most up-to-date care for the animals during the tumour implantation and growth stages, we will defer to the "NCRI Guidelines for the welfare and use of animals in cancer research" by P. Workman et al.

To deliver the best surgical outcomes for the animals and our research we will review the guiding principles listed in the "LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery 2017".

### **How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?**

We will have regular discussions with the Named Persons and animal technicians to review current approaches and whether there are any new 3Rs opportunities.

We have subscribed to the NC3Rs e-newsletter, providing monthly updates that focus on funding opportunities, 3Rs events and publications.

We will also look at attending NC3Rs events and workshops to keep abreast of 3Rs advances and approaches.

Any new advances that may impact how we work or provide new approaches will be communicated with everyone working on this project.