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NON-TECHNICAL SUMMARY

Identifying the role of the senescent cells upon the cancer and pre-malignant microenvironment.

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Liver, Cancer, Senescence, Immunity, Endothelium

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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?

Cells in the body become senescent due to damage and aging. Senescent cells (cells that permanently stop dividing, accumulate in the body and release inflammatory chemicals) accumulate within the liver at the earliest stages of cancer. We seek to understand the interaction between senescent cells, the cells that line blood vessels and cells of the immune system within the liver.

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?

Background

Cancer develops when cells in the body build up damage in their DNA (the genetic code inside every cell). This usually happens slowly, but it can happen faster if someone has long-term illnesses that cause swelling or scarring, like liver cirrhosis or inflammatory bowel disease. These illnesses can damage DNA and switch on genes that cause cancer.

In the very early stages of cancer—before we can detect it—the body has defence systems that try to stop it. One of these systems is called **cellular senescence**. In this process, cells that sense damage stop dividing. They also send signals to nearby cells to stop them dividing. On top of that, these damaged cells attract immune cells (the body’s defenders), which kill them before they can cause harm. Learning how the immune system finds and destroys these damaged cells could help us prevent cancer before it starts.

Immune cells travel in the blood to protect us from infections and cancer. When they sense a problem, like swelling or infection, they can stick to blood vessel walls and move into tissues. In the liver, the blood vessels are lined with special cells called endothelial cells, which help immune cells enter the liver to fight bacteria and viruses. We know immune cells move into the liver when cancer is present, but we don’t yet know how they respond when liver cells become senescent. We also don’t know how important endothelial cells are in this process.

If we can understand how immune cells enter the liver and kill senescent cells, we may be able to stop cancer from developing by boosting the body’s natural defences. Treatments that strengthen the immune system are already used successfully for other cancers, such as melanoma (skin cancer).

By finding out how to improve the immune system’s ability to clear these early damaged cells in the liver, we may be able to design new treatments to stop liver cancer before it begins.

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

If successful, this project will have several significant outputs:

(A) Scientific progress

The aim of this project is to increase knowledge of how senescent cells interact with the immune system (cells which attack infections and cancer) in combating the earliest forms of cancer. By the end of this project, we hope to understand how pre-cancerous, senescent cells, signal to the endothelium and the immune system to prevent cancer from developing and how this system fails.

We will aim to promptly publish and disseminate the results in publicly-available scientific journals and regular presentations of data at scientific meetings. Some data however must remain confidential e.g., when working with the pharmaceutical industry to develop and manufacture new medicines, so may not be released straight-away.

(B) Drug-development and potential collaborations with industry

Through this work, we may identify drug targets to boost the immune system in the clearance of pre-cancerous cells with the ultimate goal of developing new drugs to work in patients. Any potential drugs or drug targets that we discover would be tested in subsequent projects.

(C) Public engagement

We will use well-established routes at our institution to engage with the wider public, explaining why we believe this research is important.

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

The following groups will likely be beneficiaries of this project:

(A) Other scientific researchers

This project will directly benefit scientific researchers working in senescence, liver biology and liver cancer. Scientists will be able to access our newly developed knowledge through:

1. Publication of results in open access scientific journals and regular presentation of data at national and international meetings. This will promote new scientific work from other laboratories around the world.

2. Sharing of our study data through online databases. Generation of publicly-available data will promote discoveries by other scientists, as they will not need to repeat the same experiments in other mice if they can easily access the data they need.

(B) Future patients

Through development of knowledge about how senescent cells are cleared from the liver, we will be able to design novel immunotherapy treatments to prevent the development or progression of cancer.

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(C) Society and the economy

If we are successful and find new treatments, these will be developed in partnership with pharmaceutical companies. In addition, if we can develop new treatments for cancer, this will improve people's health and lifespan.

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

We wholly support making our published findings openly available to all. We intend to make data generated during this project open to the wider scientific community and the public, via multiple routes:

(A) Communication

New ideas from this research will be shared with scientists through talks at scientific conferences and educational talks for students. We hope that some of our findings will be of general interest and we will work with the public engagement teams at our institution to make the information interesting and digestible for lay readers.

(B) Dissemination

All the results arising from this project will be submitted to scientific journals and presented at national and international conferences.

Our published data will be deposited in publicly available databases, allowing access to our findings. Useful substances or viruses that we make in this project, will be distributed upon request or deposited in publicly accessible repositories, such as Addgene (https://www.addgene.org).

(C) Engagement

We have previously been active in explaining our work to the general public, both at fundraising events such as public tours of our institution, at science fairs, such as our institution's annual Science Festival and talks at local schools.

(D) Development and exploitation

It is possible that potential new treatments will be generated in this project. We will get advice and support from internal experts at our institution to help develop these further.

Species and numbers of animals expected to be used

- Mice: 8650

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.

Our Project

We want to study how damaged liver cells, called senescent cells, talk to the cells that line blood vessels and to white blood cells (the body's defenders). To do this, we will use mice, because they are a very useful model for studying cancer

Why Mice?

Mice are often used in medical research because their biology, especially their immune system, is very similar to humans. They also grow and reproduce quickly, which means experiments can be done in less time. Many research tools, like special antibodies and medicines, already work well in mice.

We can change mouse DNA in specific cells at certain times using well-known techniques. This can be done by injecting DNA molecules or viruses. If we want to change only the blood vessel cells, we need genetically modified mice. Our team has used all of these approaches before. For these reasons, mice are the best animals for our experiments.

1. **Senescence (Damaged cells).** We will create senescent liver cells in mice by using genetic changes or viruses. This is a standard method we have already used successfully in mice that are 6–9 weeks old. It has not yet been shown to work in other animals or in mice at different ages.

2. **Chronic Liver Disease.** We want to understand liver disease in humans. Mice can develop a similar disease. We can cause this by feeding them a special high-fat diet or giving them a chemical called carbon tetrachloride. Over 6–9 months, the mice develop long-term liver damage. This method is widely used around the world. Other models of liver disease exist, but they don't look as much like the human version.

3. **Transplantation.** To see how different cell types talk to each other, we can transplant cells into special mice that don't have an immune system. This is important because the immune system would normally attack and reject transplanted cells. Mice without an immune system can be easily bought and are often used in research. This makes the process simple and effective.

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

To manipulate the biology of senescence and chronic liver disease requires targeted genetic manipulation. This can be achieved through injecting DNA molecules or viruses, but in the case of modification of the endothelium we will use genetically altered animals. We will also use several other techniques:

1. **Liver-specific genetic alterations:** For these mice we will alter gene function within different cell types in the liver using combinations of inducing agents, viruses or drugs. These mice will be monitored by for signs of ill-health or by scans and blood tests over the course of weeks or in a small minority of mice, months.

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    1. **Drugs and medicines:** To induce some genetic alterations we need to use tamoxifen or doxycycline. Tamoxifen is a hormone-blocker and doxycycline is an antibiotic and both are used as medicines in humans. However, in mice both of them can cause a loss of appetite and weight loss

    2. **Hydrodynamic tail vein injection:** This involves the injection of genes in a large volume of saline into the mouse and results in delivery of the genes straight to the liver cells. We will use this technique to induce senescence within the liver cells before following these mice over the course of a month, or in a small minority of mice, months.

2. **Induction of chronic liver disease:** We will induce chronic liver disease by giving these mice a high-fat diet or treating them with carbon tetrachloride (a chemical that damages the liver), whilst altering liver gene function using viruses or drugs. We will follow these mice for several months and monitor them by clinical signs, imaging or blood tests.

3. **Drug treatments:** We need to test drug treatments to see if we can prevent liver disease or cell damage from occurring. Whilst we can sometimes add it to the food or drinking water, sometimes we need to give it by injection or by placing a soft tube through the mouth and into the stomach.

4. **Blood tests:** To monitor the effect of some of our experiments, we need to perform blood testing, which involves taking blood from the tail of the mouse. This takes a few seconds and involves an injection.

5. **Scans of the liver.** To monitor the effect of some of our experiments, we need to perform ultrasound or CT scans of the mice. As they need to keep still, we put the mice to sleep with a general anaesthetic for about half an hour.

6. **Transplantation:** Some mice will be injected with complex mixtures of cells that we have grown in the laboratory. These will be injected either into the skin, spleen or liver. This allows us to study these cells for prolonged periods of time and see how they interact. These mice will be monitored by clinical signs, periodic imaging or blood tests. In mice with skin tumours, we can measure the tumour size directly with calipers. For mice with liver or spleen tumours, we will monitor the tumour using imaging, such as ultrasound.

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

We are proposing a diverse plan of work that includes several procedures that the mice may find stressful. These can be divided into short-term and long-term impacts:

1. **Short-term impacts**

    1. **Handling and restraint.** Handling of the mice during our experiments may be stressful, but minimised by sensitive handling techniques. For some techniques such as blood taking or injection we have to place the mice in a restrainer for a few minutes that limits their movement and allows access to their tail. This may be stressful for a very short time.

    2. **Anaesthesia for procedures**. General anaesthesia allows us to perform procedures without the animal experiencing acute pain or stress. Occasionally, a mouse will undergo

several episodes of anaesthesia to allow us to perform imaging of the liver over time. Anaesthesia will last for less than 2-hours; it may be repeated, but not more than twice in a 24-hour period.

3. **Blood taking**. This requires restraint and injection which may be stressful and painful. This will last only a few minutes.

4. **Treatment with drugs or inducing agents by gavage.** Some substances can be given in modified feed. Some substances are given by oral gavage which involves inserting a tube into the stomach for a few seconds and might be stressful.

5. **Treatment with drugs or inducing agents by injection:** This requires handling and restraint which may be stressful for a few minutes. Some substances need to be given by injection which may be painful and sometimes need repeated dosing. We will ensure that we use the fewest number of doses to achieve our intended effect.

    1. Some inducing agents, like tamoxifen, can be painful when given by injection into the abdomen or cause weight loss when given by mouth. We will ensure we use the lowest dose and fewest number of doses required. We will closely monitor the weight of the mice receiving this treatment.

6. **Hydrodynamic tail-vein injection**: Animals are made unconscious by placing them under general anaesthetic, before intravenous injection of a large volume of saline. After the injection the mice remain lethargic for around 30 minutes, but then recover without problems in most cases. A small number of mice can die during the procedure. Mice will only undergo this procedure once.

7. **Transplantation:**

    1. **Under the skin:** We will transplant cells under the skin of some mice. This is a quick procedure that involves restraint and one or two injections. This may be painful and stressful for a few minutes.

    2. **Into the liver or spleen:** We will transplant cells into the liver or spleen of some mice. We will do this under general anaesthetic to ensure accurate placement and to minimise stress and pain for the mice. Mice will only undergo this once.

2. **Longer-term impacts**

    1. **Chronic liver disease:** To induce chronic liver disease, we will either:

        1. Give the mice a **modified high-fat diet**. The mice will get fat and so may move around less.

        2. with or without repeated **injections of carbon tetrachloride**. This involves repeated injections in the abdomen that could be stressful or painful for a few seconds.

    2. **Repeated investigations:** Some mice will undergo drug treatment or imaging several times over the course of an experiment that may increase stress over a long period. We will

limit repeated procedures to those needed to achieve our scientific aims.

3. **Tumour development:** Some mice will develop liver tumours in the longer term after tail-vein injection or dietary modification. Mice undergoing transplantation will develop tumours in the skin, liver or spleen. These animals may lose weight or develop pain, which we will treat with painkillers.

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)?

Mice	% of total
Mild	62.2%
Moderate	37.8%

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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?

When the liver is damaged, cancer of the liver can develop. Our goal is to understand how different cells within the liver interact when the liver is damaged and what goes wrong to allow cancer to develop. Crucially, we need to understand the activation of the immune system in long-term liver damage and whether the cells that line the blood vessels, known as endothelium, is involved in this process.

To understand the complex relationships between different cells in the liver, we need to study these cells in a living animal, as we cannot study these relationships using cells cultured in a laboratory. Importantly many of these changes within the liver and diseases are very slow to develop and occur over a period of weeks to months. Maintaining cultures of several types of cells in the laboratory over long time-periods is very difficult. Animal experiments allow us to perform these experiments over longer time periods.

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We can study some of these processes in liver samples obtained from patients, but these only allow us a snapshot. To manipulate or change the behaviour of specific cell types in the liver means we need to use genetically-modified animals.

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

During our research we have considered and will use non-animal alternatives:

i) Liver organoid cultures. We will make use of three-dimensional (3D) liver cell cultures such as liver- or stem-cell-derived organoids, that resemble the normal liver, where possible. These allow the study of human liver tissue in the laboratory. We can also genetically modify these organoids, replacing some of the mouse experiments.

ii) Mixed cell cultures. For some experiments we will mix two types of cells together and culture them in the laboratory, allowing us to study how different liver cell types communicate with each other. We will use mixed cultures of either pre-cancerous senescent cells or cancer cells to determine their effects upon the endothelial cells.

iii) Human samples. For experiments where we do not need to manipulate anything, we will study cell behaviour in human liver samples removed as part of patients' routine clinical care.

iv) *in silico* modelling. We will analyse publicly-available data that is relevant to our research. However, we are not aware of any computer programmes that can model the way cells in the liver communicate with each other.

Why were they not suitable?

i / ii) Organoid and mixed cell cultures. Study of cells grown in the laboratory allow us to develop ideas about which signalling pathways we will then study in living animals. However, these cell experiments do not allow the study of complex interactions, such as the immune system. This can only be studied in living animals:

a) Cell culture systems can work for two different cell types, but to study more than this is impossible, due to different growth rates and need for different culture conditions.

b) Cell culture systems cannot keep cells alive for long enough to study the effects of injury over prolonged time scales required to be representative of human chronic liver disease.

c) Introduction of the complete immune system into cell culture systems is nearly impossible due to the short-lived nature of some cells of the immune system and the very slow growth of other white blood cells that protects us from infectious diseases and cancer. To study the effect of endothelial cells upon the immune system requires a living animal.

iii) Human tissues. Studying human tissues is important to understand human health problems, but we are unable to manipulate specific cell types to see what effect these changes have. Cultures of human tissues do not survive for very long, so we cannot study disease progression. Experiments in mice allow us to do this.

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iv) Lesser species. Most research around the world on fatty liver disease and liver cancer development has been performed on mice. There is a wealth of data and experimental reagents, such as antibodies, that do not exist for species such as Zebrafish. Therefore, mice are the most suitable organism for our research. However, we will work with collaborators to develop a fatty liver disease model in Killifish to see if it is suitable to replace mice in future research.

v) Other life stages. Liver cell biology changes drastically after birth meaning that experimental findings in embryos do not reflect liver biology in adults. Therefore, we need to study adult mice in our research.

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?

For our estimated numbers, we will use:

1. around 6000 genetically-altered mice, bred within our animal facility of which ~4550 will be used in experiments and ~1450 will have the incorrect genetic modification
2. around 2650 wild-type or specific mice (lacking immune systems etc) that we will buy from commercial companies.

For the experiments we have planned, we have estimated these numbers from several sources:

1. We will work with statisticians to make sure that a minimum number of mice are used to generate significant results.
2. Our previous work. Sometimes, we have performed similar experiments which helps us to estimate how many mice are needed.
3. Previous work from others. Sometimes, other scientists have performed similar experiments which helps us to estimate how many mice are needed.

In all cases we will use the minimum number of animals required, for the experiment to give us useful data.

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

When we plan experiments, we always think about the 3Rs (Replace, Reduce, Refine) and look for ways to improve. We reduce the number of mice we use by:

1. **Careful planning.** We use special tools, like the NC3R's Experimental Design Assistant, to make sure our experiments are well designed. We also use methods like randomisation and blinding (techniques that minimise any bias that could affect our data), so our results are reliable and not just chance.

2. **Small pilot tests.** Before doing big experiments, we run small tests. This helps us check that our tools work properly and shows us whether the effect we are looking for is small or large. That way, we know the right number of mice to use and don't waste animals.

3. **Better techniques.** We use modern tools, like viruses and genetic editing, to make changes directly in the mice we need. This avoids breeding lots of extra mice with the wrong changes.

4. **Getting the most from each mouse.** We collect as much information as possible from every animal. This includes repeat scans while they are alive and studying many organs after. We also share data and samples with other scientists so that experiments don't need to be repeated.

5. **Following standards.** We follow international rules like the PREPARE and ARRIVE guidelines. This means our work is done to a high standard and reported clearly, so other researchers do not need to repeat it.

6. **Not repeating old work.** We carefully read existing studies before we start. If someone has already done similar work, we contact them to see if we can use their samples, which saves using more animals.

By taking these steps, we make sure we only use the minimum number of mice needed while still answering our research questions.

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

We will use new techniques that will allow reduced numbers of mice to be bred for use in experiments. Complex breeding programmes always generate large numbers of mice with the wrong genetic modification. Our techniques allow specific genetic modification of liver cells and include:

1. **Hydrodynamic tail-vein (HDTV) injection.** We currently use this technique on a regular basis, which allows delivery of small sections of DNA to liver cells, where it is cut and pasted into these cells DNA, altering their behaviour. Using this method we can take any mouse and express any gene that we have designed in the laboratory, within the liver.

2. **Adeno-associated viral (AAV**) **injection.** These viruses home to the liver, again allowing gene delivery to liver cells. This strategy is complementary to the HDTV injection technique, but only allows certain small genes to be delivered to the liver.

3. **CRISPR-based DNA editing**. This novel technique allows us to use either HDTV or AAV injection to deliver molecular machines (Cas9 enzymes) allowing us to directly edit the DNA in

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liver cells. Therefore, this technique allows us to create specific mutations in the liver cells in a live mouse.

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.

We will use the most refined techniques available to study the interaction between senescent cells and blood vessel cells in liver disease. We will use the following techniques:

1. **Modifying the DNA of liver cells.** Using either hydrodynamic tail-vein (HDTV) injection or injection of adeno-associated viruses (AAVs) will allow us to directly deliver and express different genes in liver cells. These techniques mean that we can use wild-type mice and do not need to generate and breed lots of types of genetically-modified mice. This also reduces the overall numbers of mice needed for our project.

2. **Induction of chronic liver disease.** To study liver disease, we will use non-invasive techniques that are used in many laboratories around the world, which minimise pain and suffering. We will induce chronic liver disease (CLD) in mice by:

    1. Modifying the diet of the mice to include lots of sugar and fat. These modified diets will lead to chronic liver disease, that looks like human fatty liver disease within 6-9 months.

    2. Treating the mice with liver-damaging drugs. This treatment leads to liver damage and over several months the mice will develop chronic liver disease, that looks like human liver cirrhosis.

    Previous experiments by ourselves and other scientists have shown us the doses and time-scales of these treatments that result in liver disease. This allows us to plan study of the mice at specific time-points before the mice develop liver-related problems.

3. **Liver-specific genetically modified mice** will allow us to study particular genes or pathways, particularly at specific times. Where possible we will use inducible DNA modifications to specifically target particular tissues and limit the effects on the mouse. This will be particularly important to limit our modifications to the liver and cells that line the blood vessels. This approach means that problems that might develop if we altered the DNA in the whole mice are avoided.

4. **Liver imaging.** We are able to perform CT and MR scans of the liver to study the development of liver disease over time. This means that we do not need to perform painful procedures such as liver biopsy or liver surgery to obtain the information that we need.

Why can’t you use animals that are less sentient?

Mice develop liver disease, that looks very similar to liver disease in humans, but it occurs much more quickly. Lower species such as flies or worms do not develop chronic liver disease and do not have immune systems like humans. Indeed, their body organs are so different to humans that they are not suitable to study. Mice permit us to study senescence, chronic liver disease and the role of all components of the immune system. Many suitable genetically modified mice already exist and if they don't the tools are available to create them. Therefore, mice represent the most appropriate animal model for our experiments.

We are unable to study earlier stages of development as:

1. We are interested in senescence or pre-cancer. This has not been demonstrated or produced in embryos or juvenile animals in previous scientific studies.

2. Induction of senescence or chronic liver disease takes time, which would result in the animals being adults by the time that chronic liver disease had developed.

We are unable to perform experiments under long-term anaesthesia as senescence takes days and chronic liver disease weeks to develop. Animals cannot be kept under anaesthesia for long periods as they would become dehydrated and develop complications of anaesthesia, such as infections.

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

We will seek to constantly improve the welfare of the mice within our colony.

1. **Staff development.** Handling of the mice work will be performed by experienced doctors, scientists and technicians who will closely monitor the mice. A key refinement is ensuring that staff performing husbandry or experimental techniques are fully trained. This has been the key in our current facility to improve the success rate of delivery of hydro-dynamic tail-vein injection.

2. **Housing and husbandry.**

    1. **Environmental enrichment.** Environmental enrichment to promote normal animal behaviour is standard in our mouse facility, including wooden chew sticks, nest-building materials and fun tunnels.

    2. **Non-aversive handling.** Where possible we will use non-aversive handling techniques, such as cupping or lifting in tunnels, rather than picking up by the tail, to reduce stress.

    3. **Avoidance of solo housing.** We will plan to house the mice together where possible to avoid loneliness.

    4. **Acclimatisation.** Any mouse that is brought into our colony will be given one week to get used to our mouse facility, before commencing any experimental procedures.

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3. **Improved experimental design.** Pilot experiments will be performed when we try new techniques or models, allowing us to identify any unexpected problems and improve the full experiment. This approach will also mean that we will only use animals that are absolutely necessary.

4. **Improved techniques**. We set strict limits to minimise the harm or suffering to the animals used.

    1. We will only use a limited number of **surgical procedures** and ensure that pain is treated after these procedures.

    2. We will optimise our techniques to minimise suffering, including:

        1. Only using single-use needles so that they are sharp for every procedure.

        2. Warming fluids for injection to body temperature, so we do not cause hypothermia in the mice.

    3. We will use **general anaesthesia** for:

        1. Non-invasive imaging, allowing us improved pictures of the liver.

        2. Hydrodynamic tail-vein injection which is expected to increase the success rate of this technique

    4. Our institution has developed **Standard Operating Procedures** for use of substances, like tamoxifen, to ensure best practice and promote the welfare of the mice receiving these.

    5. **Close monitoring** during longer-term experiments will ensure that animal suffering is kept to a minimum and clinical signs are picked up and addressed promptly.

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

We have extensive experience in studying senescence and chronic liver disease in mice. We will use this experience during this new project, but will make use of published guidelines to improve our experiments.

1. We will improve our experimental design following the **OBSERVE guidelines** (Nature Protocols, 2024) and using tools, such as the **NC3Rs experimental design assistant**.

2. Our animal experimentation programme will be run according to the guidelines and position statements from the **Laboratory animal science association (LASA).** We will care for our mice according to the guidelines laid out in the **Workman et al** (British Journal of Cancer, 2010) and **OBSERVE guidelines** (Nature Protocols, 2024)**,** on animal welfare in cancer research.

3. During our experiments we will collect data on mouse phenotype and clinical signs according to the **FELASA guidelines** and use standardised scoring sheets to monitor the overall and changing condition of our experimental mice.

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4. When reporting our experimental results we will adhere to the **ARRIVE guidelines**, aiming to provide a comprehensive description of experiments and findings.

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

During this project I will ensure that I keep abreast of the latest developments in the 3Rs through several sources:

1. **Continuous professional development.** I will attend relevant training courses to keep my licence current, that will include developments in the 3Rs.

2. **Institutional 3Rs search tool.** Our establishment has a web-based search tool that allows me to keep up to date with the 3Rs.

3. **Online tools.** There are a number of websites that we will use to keep informed:

    1. **Animal welfare**: Organisations that promote best practice in animal welfare, such as the National Centre for the 3Rs (www.nc3rs.org.uk/) or the Norwegian National Consensus platform (norecopa.no/).

    2. **Alternative model systems.** Papers published on the Alternatives to Laboratory Animals journal website (journals.sagepub.com/home/atla).

4. **Peers and colleagues.** In biomedical research I have extensive connections with other researchers throughout the UK and the world. I attend numerous conferences, where new findings are presented, allowing improvements in our techniques and experiments.Our facility also has two Named Information Officers (NIO) who will be a valuable source of information about new techniques in animal research.

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