Home Office

NON-TECHNICAL SUMMARY

Remodelling of stem cells and niche cells: from development and regeneration to disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Respiratory system, Stem cells, Tissue repair, Ageing, Lung disease

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant, aged

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?

The overall aim of this project is to investigate how the cells of the lung adapt to tissue perturbations (situations driving the tissue away from normal health condition) such as ageing, cell depletion and tumour development. I aim to learn if we can use this information to improve injury repair in lung tissues.

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?

The lung is exposed to external environment and faces with constant challenges by various insults such as allergen, air pollution, chemicals, and pathogens which lead to tissue damage and ultimately developing lung diseases. Lung disease is one of the top three killer diseases in the worldwide. The Asthma and Lung UK estimates that approximately 12.7 million people in the UK have a history of longstanding respiratory illness including lung cancer and pulmonary fibrosis (scarring of the lung tissue). Most significantly, the recent COVID-19 pandemic, caused by SARS-CoV-2, has already recorded more than 300 million confirmed cases, while as of 18th January 2022, more than 15 million deaths have been reported worldwide (WHO, 2022). The cells of the lung are the main target of SARS-CoV-2 infection. Further, patients with chronic lung diseases are at greater risk of developing severe COVID-19, presumably due to the unresolved lung damage or alteration already present within their lungs or an overactive immune response. Therefore, with the global scale and clinical burden of COVID-19, a deeper understanding of lung repair mechanisms following tissue perturbation (situation driving the tissue away from normal health condition) is urgently needed.

In this project, we will study the way in which the cells of the lung function under normal conditions, evaluate how their behaviour changes in response to tissue perturbations. By addressing these questions, we expect to understand how cells behave in different contexts, and whether this information can be used to change their behaviour to improve the health condition of the lung. We ultimately aim to improve lung injury repair with minimising fibrotic scarring (regeneration) and to treat disease more effectively. By investigating cellular changes from its early stages of lung disease, we expect to identify markers that can assist in the early detection of these diseases, improving the long-term prospect of those patients. We anticipate that our work will identify targets of potential medical relevance that will contribute towards solving the challenges being currently faced in the clinic.

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

The main output of our work will be in the form of scientific publications that will be of relevance to other scientists in our or other related research fields. These publications will provide novel information on the processes regulating how the cells of the lung respond when not in normal health.

In our publications, we aim to cover a number of relevant aspects:

1. Identify changes in the damage repair ability of cells during aging, and its implication in chronic lung disease.

2. Uncover how changes in the immediate environment that surrounds stem cells affect tissue damage repair.

3. Define how cells return to an early developmental stage when perturbed, and its implication in lung cancer.

As standard practice, the data generated in our work will be made publicly available in free repositories to ensure that it can be used by other researchers and facilitate scientific discovery beyond our own studies. This should reduce repetition of the same work by other laboratories, decreasing the number of animals needed for related research purposes.

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

Our overall challenge is to identify the processes controlling the ability of a lung tissue to heal (regeneration) and to develop lung diseases including lung cancer and pulmonary fibrosis (scarring of the lung tissue), and translate this knowledge into therapies that benefit the patients and healthcare providers. For this, the immediate aim is to understand how the cells deviate from normal behaviour when they face an injury/damage or develop disease. Then we need to determine which molecular mechanisms regulate the cellular response. Our ultimate aim is to determine whether we can intervene in these processes, using drugs, in order to take cells back to their normal healthy state.

In this programme of work we will address these important questions. In the short term, new information that comes from this study will be presented through publications and conference presentations, or shared with organisations such as the Wellcome Trust and The Royal Society. This will mostly benefit the scientific community, particularly those with interests in tissue regeneration and chronic lung disease. In the long-term, our findings could be used to devise new strategies for the early detection of lung diseases as well as to develop novel therapies to improve lung regeneration and treat diseases. This will benefit patients suffering from declining lung function, professionals in the healthcare community, as well as companies with an interest either in i) experimental systems to study disease or in ii) in the potential identification of novel drug targets.

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

We maximise the outputs of our work by doing the following:

- To increase the outputs of the work conducted within this study, the management of individual project is closely supervised by me. This way I avoid effort duplication and ensure that the resources are maximised and shared between members of my laboratory, where feasible.
- By disseminating our observations from early stages, for example via conferences, we create new collaborations with experts in research fields beyond our own. They positively contribute to the progress of ongoing projects and ensure their completion to the highest standards. This has previously allowed us to establish collaborations with researchers interested in samples created as a side-product of our research. For example, samples are currently being shared with other

groups examining the cell behaviour in other organs such as the intestine, brain, liver, among others.

- Moreover, in our effort to understand the behaviour of cells, we conduct the analysis of large-scale datasets. To maximise the outputs obtained from these complex datasets, we collaborate with experts in theoretical physics, mathematical modelling, and computational analysis, who have the skills to develop theories explaining the results obtained in our experiments. Methods and datasets generated in these types of analyses are made publicly available as part of the relevant research articles enabling new collaborative projects.
- Our work will be published in free, public repositories online and in international scientific journals. In our publications, we do not only include positive data, but also experimental results that fail to support our hypotheses. This also prevents duplication by ensuring that other laboratories do not spend resources in ideas that have already been tested.
- We will share our work with the public via public engagement activities. This will allow people to learn about novel aspects of regenerative medicine and lung diseases, and will provide the opportunity for a productive exchange of ideas between patients and scientists.

Species and numbers of animals expected to be used

- Mice: 42050

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.

The mice will be used in this project for tracing the changes in behaviours of the lung cells under tissue challenges (conditions away from healthy normal lungs). A crucial reason for using mice is that cellular responses to tissue challenges represent a complex biological process that involves numerous cellular types, signalling pathways and environmental factors. These processes can differ substantially in organisms other than mammals and, specially, in invertebrates. Mice are the smallest species with lowest susceptibility to neurophysiological conditions (situations that affect both physical and conscious functions such as stress, anxiety, and mental activity) that have mammalian lungs. Moreover, stem cells that contribute to lung tissue maintenance have been extensively studied by using multiple mouse models that are readily available and capable of reproducing the human settings. Critically, at the moment, there is a critical limitation to investigate the time-course changes of disease initiation and progression for which human samples are typically unavailable yet mouse disease models are allowed. Our study will focus on the early sequential changes of lung cells upon tissue challenges using mouse models which also allow relatively easy genetic manipulation and tracking the changes of individual cells over time.

We will use mice that undergo different stages of their lifetimes (from birth to aged stages) because we are interested in understanding why we exhibit a different repair response and disease incidence as we age.

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

Most of our experiments will use genetically modified animals that allow us to follow the individual cells in which we are interested. This will facilitate the tracking of individual cells to study their behaviour in response to perturbations (such as ageing, genetic mutation, or cell ablation). To address our research questions, animals will typically undergo one of three main types of experiments:

1. Ageing: Animals will be administered various substances to label their cells, by delivering them into their stomach. This will help us track the behaviour of cells at different time points throughout the lifetime of an animal. Samples will be collected after humanely killing mice at different ages from birth and up to 24 months of age. The labelled cells will be analysed to investigate the impact of ageing on cells.

2. Genetic mutation: Animals will be administered substances to alter genes that are associated with lung cancer, by delivering them into their stomach. Animals will then be allowed to develop tumours in the lung. Since we are interested in early tumour formation, animals are humanely killed before showing any signs of distress or suffering. Tumour samples will be collected at different times to capture the point at which tumours form. The cells will be analysed to investigate how tumours emerge.

3. Cell ablation: Animals will be administered well-studied substances for local cell ablation in the lung, by delivering them into abdomen or lung. Alternatively, animals will be exposed to targeted radiation aimed at the lung while sparing the rest of the body. The procedure of lung delivery will be completed under inhalation-mediated anaesthesia for less than 5 minutes so that animals remain in a state of sleep/unconsciousness during the procedure. Some animals may develop mild scar (fibrosis) after cell ablation in the lung. Some animals may be treated with additional substances to label the cells and track their behaviours during replenishment process of lost cells or development and progress of lung fibrosis. Aged animals may be used to compare these features to the young animals. Subsequently, samples will be collected after humanely killing mice at different stages of replenishment or fibrosis to investigate the sequential changes in cell behaviour. These experiments will shed light on how tissue repair changes as animals age.

For any of these typical experiments we may sporadically treat a small number of animals with drugs that will help us discover the processes that regulate cellular behaviour.

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

Please note the adverse effects presented here are based on our previous experience using the proposed techniques. Our work will be conducted in a way that will not lead to long-lasting pain, suffering or distress and have no lasting effect on the animal’s health and wellbeing. The genetically modified mice used are not expected to show deviations from normal health.

All animals are carefully checked regularly and if there are any concerns animals are examined and weighed. The majority of the animals will be used in procedures under which they suffer either no, or mild, adverse effects. For example, transient pain or discomfort following an injection. A smaller number of animals (less than 5% of the total) will be exposed to procedures of moderate severity to model different aspects of human lung disease or treatment. Some animals sporadically present transient weight loss of up to 15%, which is usually recovered within the next 2-12 days. In some instances, animals become sick from unresolved tissue damage or scarring that causes sustained altered breathing rate and subdued activity, in which case they are humanely killed.

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.

-Mild: 67%

-Moderate: 33%

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

- Killed
- Used in other projects

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?

Animal experimentation is required to obtain relevant information on cellular behaviour within the living organism. In order to identify treatments of relevance to improve injury repair and the symptoms of illness, we need to understand the way in which different cell types interact with each other. The lung is complex organs formed by different layers and tissue compartments that contain different types of cells. In response to an injury or genetic alteration leading to lung disease, all these cells need to communicate in order to elicit a coordinated response that results in injury repair or disease development, respectively. Unfortunately, this complex communication network is something that so far cannot be fully explored merely by growing cells outside the animal. Additionally, most techniques that work with cells outside of living animals do not fully reflect their normal behaviour over long periods. Hence, animal work is necessary to disentangle how different cells communicate and cooperate in health and disease; an aspect of critical importance to understand the basis of tissue regeneration and cancer.

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

My laboratory is a pioneer group in developing methods, called three-dimensional (3D) organoid systems, to grow tissues outside the animal. These platforms recapitulate physiological features of lung tissues in a dish and thereby significantly replace injury/disease animal models that generally employ moderate to severe animal protocol. Additionally, we have published the details of our methods which can be used by other scientists who can also replace their animal models. Importantly, we have been educating more than 500 scientists from academia and industry to grow 3D lung organoids in a 2-day workshop. Whenever possible, we will implement this technique to obtain preliminary data to inform experiments with animals in this project.

Why were they not suitable?

Tissues are formed by many different types of cells. We are interested in understanding how those different types of cells interact with each other and contribute to ageing, injury repair and disease development over long periods of time. Unfortunately, to date, by growing cells/tissues outside the animals, we are not able to fully mirror the complex cellular interactions and time-course long-term changes in cell behaviour that take place in the animal. Until then, experimentation with animals will still represent the gold standard to unveil these intricate processes.

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?

The predicted number of animals needed for this project has been based on the following:

1. Animal numbers have been worked out with the advice of expert statisticians. This ensures that our experiments are designed properly and that the results obtained have enough statistical power to draw meaningful biological conclusions.

2. Animal numbers have been calculated based on the animals used in my laboratory in similar work over the past 5 years. I have also accounted for an increase in the number of members that form my laboratory, something anticipated for the next five years.

3. We have carefully considered the best way to make sure we keep the lowest number of different types of mice (strains) for breeding, while ensuring we have enough mice to use for experiments.

4. Test experiments, called pilot experiments (which use a smaller number of animals; typically 3) will be used to calculate the amount of a substance that we can safely inject into the animal. It is important to find the smallest amount of the substance that has an effect but that will not harm

the animal in any way. This will then allow us to perform the intended experiment using only enough mice to be sure that the results we obtain are statistically significant.

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

We have taken advice received from a local, qualified expert in statistics. This will make sure that each experiment produces statistically meaningful data. Where possible, all experiments are designed to get the most information using the least number of animals possible. We will also take into consideration the NC3Rs guidance and experimental design assistant tool (https://www.nc3rs.org.uk/experimental-design-assistant-eda; https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction). Additionally, all experiments will be designed taking into account the PREPARE guidelines (a document that gives scientists advice on how to plan animal research and experiments).

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

We implement different measures to reduce the number of animals used. Among them:

- Efficient breeding techniques: We will breed our genetically modified mice in a way to minimise waste. Any types of genetically modified mice (strains) that are not being used for scientific studies will be frozen as embryos for future use.

- Experimental design: Critical experiments are designed in collaboration with experts in statistical physics. With their assistance, we determine the number of experimental animals required to answer each of our scientific questions.

- Mouse strain management: animals are bred only to fulfil our experimental needs. However, any unavoidable excess of animals is used for in vitro experiments (culturing cells outside the animal), validation or pilot studies (as long as they are compatible). The latter represents a critical consideration in our laboratory, and allows us to ensure that mouse waste is minimised as much as possible. While this approach requires a significant amount of coordination and team effort, it is essential to reduce the number of animals we breed or purchase. We will also take into consideration the NC3Rs Breeding and Colony Management Resource (https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management).

- Animal and tissue sharing: I have a network of collaborators within the UK who work in my or a similar research field. We exchange particular types of mice (strains) between us to reduce animal imports, the need for additional breeding and its associated excess. Similarly, to increase the output of our work, we share tissues with members of other research groups and collaborators who examine different areas of the body (tissues), such as the liver and the intestine. Additionally, we are registered in the animal tissue sharing list of our institution.

- Growth of tissues outside the animal (3D tissue cultures): Using this method, one single lung can produce sufficient cells to perform at least 3 experiments instead of one. Thereby, this system effectively reduces the number of animals used by two thirds. However, as indicated above,

studying interactions between different types of cells remains a limitation when using this technique.

- Mathematical modelling: By working with our collaborators, we use our data to develop mathematical models that explain how cells behave and interact with each other. The created hypotheses are then tested in the laboratory. This makes our science more targeted, which allows us to significantly reduce the number of animals needed to understand how cells work.
- Advanced imaging and molecular methods: Dissected samples are analysed using state-of-the-art whole-tissue imaging and molecular techniques that require minimal tissue material to obtain meaningful results.
- We will also consider subscribing to ATLA (Alternatives to Laboratory Animals) published by FRAME (Fund for the Replacement of Animals in Medical Experiments), in association with SAGE publishing, featuring articles on the latest research relating to the development, validation, introduction and use of alternatives to laboratory animals.

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 genetically modified (GM) mice to carry out our plan of work. Most of our GM mice bear genetic alterations that allow us to label cells and track their behaviour in the tissue. Hence, the majority of genetic alterations we propose are not expected to lead to any adverse effects, except animal models for early tumour formation (please see below).

The animal procedures proposed in this study will inflict minimal pain, suffering or distress to mice:

- We administer substances to mice to label and track cells over time. These methods are based on previous studies and our long-term expertise using them. We use the lowest dose needed to observe an effect. Hence, we expect our treatments to cause no harm to animals.
- We also use GM mice bearing lung cancer-associated genes to understand how cells work and explore the earliest stages of tumour formation in the lung. Our project mainly focuses on the early tumour development and use well-established GM model system based on our experience and published literature. Therefore, mice rarely show complications apart from developing discrete lesions, and will be killed before the tumours have an impact in any vital process.
- Animals receive drugs causing localised short-lasting damage in the lung. These methods affect the limited region of the lung and ablate specific cell types which are typically replenished within

1-2 weeks, depending on cell types and methods. Therefore, effect is both refined and only short-lasting. Animals undergo inhalation-mediated anaesthesia which allow a rapid recovery (less than 5 minutes) from procedures and unconsciousness during the procedure.

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

In our studies, we investigate how different cell types function under normal circumstances, and how they change their behaviour in response to injury and early disease development. Given the differences observed in injury repair and incidence of lung disease, such as lung fibrosis and cancer, as we age, it is important to investigate cell behaviour over long periods of time and at different stages throughout the life of animals (from birth to aging). Unfortunately, these long-term experiments require living organisms, limiting the use of other less sentient animals such as anaesthetised mice or tissues grown outside the animal.

Another critical aspect of our work focuses on investigating how different cell types interact and impact on each other's behaviour. For these studies to be clinically relevant, they need to be performed in mammalian animals, closer to humans. Results in lower species such as fish, worms or fruit flies, would be of very limited medical relevance.

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

All methods in this project will use techniques that reduce animal stress and make sure the animal does not suffer as detailed below:

General:

- We will make sure to use the best care methods to improve the quality of life for the animals. Mice will be placed in cages and will be provided with nesting and bedding material to stimulate mouse activity and provide them with shelter if needed.

- Side effects from procedures will be monitored by regularly weighing animals, daily health checks, and the use of scoring sheets, as required. This will prevent animal suffering. Animals showing any signs of suffering will be immediately killed. This is called the Humane Endpoint. We are not expecting animals undergoing procedures under this licence to experience suffering. If an animal does begin to look unhealthy, we will monitor it more frequently and provide pain relief if needed. If it does not improve the symptoms of illness the animal will be killed by a humane method.

- Some substances have to be given to animals by injecting them in specific places. We have therefore asked to be allowed to use different administration routes (e.g. intraperitoneal [injection into the abdomen], oral [via the mouth] and intratracheal [into the lung] in this project). We will always use the least harmful route possible to give an animal a substance. This is to make sure we cause the smallest amount of discomfort or pain to the animal.

- All animals that are brought into the animal facility will be allowed at least 7 days to get used to their surroundings. This process is called acclimatisation. We will also allow them to get used to

the animal technicians prior to use. This will reduce the amount of stress the animal experiences and will improve their well-being.

- To improve the quality of life of our animals, we always house them in groups, unless strictly necessary due to experimental reasons or unexpected husbandry issues such as fighting. Animals have enrichment in their cages (such as wood sticks, bedding and nesting material) for extra comfort and enhanced mental and physical health.

- We will carry out pilot studies when new substances, radiation doses, or genetically altered (GA) mouse lines are used for the first time. These preliminary experiments will be guided by the relevant literature. Conditions are first validated in reduced cell culture pilot studies using less than 3 animals to perfect the experimental design required to satisfactorily address a particular experimental question.

Surgery procedures:

- Surgery will be carried out in a clean manner (using aseptic technique). We will make sure to meet the level set out in the Home Office Minimum Standards for Aseptic Surgery and the LASA Guidance on Preparing for and Undertaking Aseptic Surgery (2017).

- We will ensure that animals suffer as little as possible during surgical procedures by giving the animals medication to manage their pain under the advice of the Named Veterinarian Surgeon. We will also consider the use of medicated palatable substances for voluntary treatment such as flavoured jelly, paste or milk shake liquid.

- Animals will be monitored before and after surgery to ensure that any deviation from normal health is picked up. Observations will be monitored on a chart. Animals will receive additional pain relief medication as needed, and advice from the Named Veterinarian Surgeon will be sought if animals show any deviation from normal behaviour.

- Surgical procedure will be completed under inhalation-mediated anaesthesia for less than 5 minutes so that animals remain in a state of sleep/unconsciousness during the procedure.

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

We follow the guiding principles on good practice for Animal Welfare. Our experiments are planned following the "Planning Research and Experimental Procedures on Animals: Recommendations for Excellence" (PREPARE) and "Animal Research: Reporting of In Vivo Experiments" (ARRIVE) guidelines. We attain to the LASA (Laboratory Animal Science Association) guidelines, as well as the NC3Rs published strategy for improving animal welfare (see publications details below):

- Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/laban.1217

- LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.). http://www.lasa.co.uk/publications/

- Smith D, Anderson D , Degryse A, Bol C, Criado A, Ferrara A, Franco NH, Gyertyan I, Orellana JM, Ostergaard G, Varga O, Voipio H (2018) Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. Lab Animal 51(1S): 5-57. doi: 10.1177/0023677217744587

- Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T (2018) PREPARE: guidelines for planning animal research and testing. Lab Animal 52(2): 135-141. doi: 10.1177/0023677217724823.

For surgical procedures we follow the Laboratory Animal Science Association (LASA) Guidance on Preparing for and Undertaking Aseptic Surgery (2017) and the Home Office Minimum Standards of Aseptic Surgery.

For the breeding of genetically altered mice, we will follow the guidelines provided by the Home Office and the NC3Rs Resources on 'Genetically altered mice' detailed in:

- https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf

- https://www.nc3rs.org.uk/GAmice

Additionally, for cancer studies, we will refer to:

- Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double AJ, Everitt J, Farningham DAH, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA, Committee of the National Cancer Research Institute. Guidelines for the welfare and use of animals in cancer research (2010). Br J Cancer 102(11): 1555-1577. doi: 10.1038/sj.bjc.6605642.

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

At our institution, we count on with the outstanding support of our Biofacility Service. They keep us informed (through their central team) about new developments on 3Rs and offer us expert advice on how to implement them in our ongoing studies (via our very experienced team of animal technicians, Named Animal Care & Welfare Officer (NACWO), Named Veterinary Surgeon (NVS) and Named Information Officer (NIO) all of them experts in animal experimentation).

We follow the website of the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs, available at https://nc3rs.org.uk/resource-hubs). This allows us to stay up-to-date on the most relevant information.

We also use our Institutional 3Rs search tool. This contains an up-to-date database with information on the best ways to reduce or replace animals in our experiments. It also contains advice on how to refine methods in order to reduce animal stress.

Additional guidance and information on the most appropriate and refined techniques for our studies may be obtained from external sources, including:

- Laboratory Animal Science Association (LASA) Institute of Animal Technology (IAT)
- Norecopa (https://norecopa.no/databases-guidelines) Relevant literature
- ARRIVE Guidelines