Home Office

NON-TECHNICAL SUMMARY

Zebrafish models of inherited neurological diseases

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

inherited neurological disease, brain, muscle, disease mechanism, treatment

Animal types	Life stages
Zebra fish (Danio rerio)	embryo, neonate, juvenile, 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?

Our overall aim is to examine in zebrafish animal models how some changes identified in the DNA of patients lead to the development of some rare neurological diseases affecting the brain, peripheral nerves and skeletal muscle.

In addition to investigating the disease mechanism of these rare inherited neurological conditions, we will also study the effect of potentially beneficial treatments in our zebrafish models.

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?

We study some rare childhood onset severe inherited neurological diseases, called mitochondrial disease. Mitochondrial diseases affect 1 in 5000 people, they affect the brain, skeletal muscle, peripheral nerves, heart and liver, leading to severe disability or death in children. In these diseases the brain, the nerve cells and the muscles don’t function properly, and patients have difficulties with movement and coordination. The cause of the diseases lies in the DNA of the patients, which is the material in the cells of all living things that contains the information for their characteristics and appearance. These diseases are currently incurable and have a devastating impact on patients and their families as well as requiring substantial resources from the national health service. The lifetime treatment cost for a patient with serious mitochondrial disease is around £1.3M (NHS, 2016).

In our laboratory we use human cellular models (cells from patients) to study these rare neurological conditions, however, for understanding how the gene affects the whole organism (a person) we need animal models (an animal that represents the patients` condition) as we currently cannot fully recreate a representative biological system using only human cells. Further, it is currently required by regulatory agencies to trial the effect of treatments in animal models before giving them to patients.

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

Our aim is to better understand the disease mechanisms in fish, which will help us to define which treatment can be beneficial. This informatiion will contribute to the development of new and improved treatments for patients in the long term. We also aim to test drugs for their ability to correct defects in fish, which will inform drug development programs for patients with mitochodnrial disease. Our aim is to provide better and effective treatments for patients with mitochondrial disease. We also plan to publish our results in scientific journals, so that other researchers can learn about our findings to help move treatments closer to reaching patients.

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

The major short term benefit of this research is to the scientific community by progressing our understanding of how changes in specific parts of the DNA lead to a neurological disease. For example, if we can show that the same mutation (change in the DNA sequence) in a gene of a patient also results in zebrafish showing similar disease characteristics, we can use this information to indicate that mutations in this gene are responsible for the patients disease. When we recreate mutations in fish, we observe if they have a muscle weakness or other typical symptoms seen in the patients. This helps link the patients disease to a genetic cause.

Longer term benefits will be that our research will generate new and better treatments for patients with mitochondrial disease. The information will guide clinicians treating patients and will be also useful for scientists in the field. This will result in improved diagnosis and treatments of patients. For example, it has been indicated that in patients with certain mutations that lead to lower amounts of mitochondrial DNA (the DNA inside mitochondria) may respond to treatment with nucleosides (DNA building blocks). If we can show that the fish with the same mutations respond to this treatment, this may inform the clinicians treating patients that this is an effective treatment.

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

We will publish our data in open access (free for anyone to read), peer reviewed (reviewed by other reputable scientists) journals and present at national and international scientific conferences. We widely collaborate with clinicians and scientists world-wide on this research to increase the success of our work and also to enable very specific experiments in collaboration with our partners.

We try to maximize the success of our research by following the most advanced technologies. In the event some approaches are unsuccessful, we have the knowledge and experience to troubleshoot, or use alternative model systems. In our lab we also work with human cellular models.

In the cases that we have negative results or unsuccessful approaches to the research, we aim to make this information known to the scientific and wider community. This can be done via communications in group meetings/conferences or in publications, making these important negative results available to as wide an audience as possible.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 25500

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.

Muscle, brain, heart and eye are the organs affected by these neurological diseases early in life. These are complex organs made up of multiple cell types, which means that cell culture models that generally

consist of a single cell type, have limited applicability to patients. Therefore, many pre-clinical studies (research performed before treatments reach testing in patients) can realistically only be achieved in whole animals. Zebrafish are a good model system as they share over 80% of human disease genes, meaning there is a high chance that we can study the same human disease gene in zebrafish. While zebrafish are not mammals, they are vertebrates, sharing many of the organ systems and tissues we are interested in studying. In addition, the small size of larval zebrafish allows us to perform high throughput analysis (the ability to study lots of samples in a short timeframe), which allows for statistically robust data, something which is more challenging to achieve in higher organisms.

We plan to generate genetically modified zebrafish carrying the same changes in the DNA as identified in patients and investigate how these changes impact the development and function of the different organs. Primarily, we plan to do most of the research on zebrafish less than 5 days post fertilisation (dpf), as most of the organs of interest are developed by this stage. However, based on our recent data, sometimes we need to work on slightly older, but less than 14 dpf fish to study the effect of some treatments. This is due to how the diseases manifest, as they only start to show signs of disease after all the cells have finished rapid development, which is usually around 5 – 7 dpf. So to fully understand how the mutations affect the fish, sometimes we need to study them up to 14 dpf.

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

When drug tests are performed on zebrafish, the substances are administered by dissolving them in the tank water for up to a maximum of 3 months but more typically for 14 days, which is naturally ingested. No invasive methods are required, minimizing the stress for the animals during the experiments. This is one of the big advantages of using zebrafish to test potential drug treatments. Most animals will be humanely killed at the end of the experiments except those required for breeding who will be expected to be only mildly affected, if at all.

The only non-experimental surgical procedures performed on zebrafish would be the caudal fin biopsy (taking a small amount of the tail to determine if a fish has a mutation). This is a routine procedure that is well established, although there are also options to perform alternative, non-surgical, methods (swabbing) which we may explore.

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

The experiments that we undertake involve the use of animals as models for human neurological diseases and so the effects on the animals in part reflect those diseases. However, we will limit the effects on the animals to the first 5 days of life (embryonic and early larval period) wherever possible. During these first 5 days, zebrafish are believed to have less capacity to experience suffering and are not considered protected animals by the Home Office legislation.

Some of our zebrafish models failed to inflate their swim bladder (an air filled organ, which helps the fish to maintain their position in the water), which may be associated with different genetic defects. This defect prevents the animals from normal swimming behaviour however, this does not cause the animals any additional suffering. It is necessary to keep the fish longer than 5 days in some of the experiments we do to determine the full consequence of mutations and effect of treatments. When treating fish longer than 5 days, we will need to withdraw feeding up to 14 days, which has been shown

to not cause any additional harm to the animals, as has been previously shown by our colleagues. We will not perform food withdrawal beyond 14 days.

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)?

Zebrafish: Mild 100%

Moderate 0%

Severe 0%

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?

Muscle, brain, heart and eye are the organs affected by the diseases we are studying; all of these are very complex organs made up of multiple cell types. This means that cell culture models, which generally consist of a single cell type, have limited applicability to patients. Therefore, many pre-clinical studies can realistically only be achieved in whole animals.

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

Where possible, we also use primary cells derived from patients in parallel to investigate aspects of the diseases. In the last few years we have started to utilise stem cell derived organoids, particularly brain organoids, to investigate the diseases of interest. These allow us to go beyond simple primary 2D cell monocultures and investigate how mutations affect neurodevelopment and neuronal function in 3D models that more accurately represent the human brain. We also plan to start trialing therapies in these organoid models. Utilization of organoids has resulted in less reliance on animal models and they now make up the large majority of the research we do.

In addition, if muscle samples become available from patients, we will use this tissue instead of animals to study the consequences of the disease in this tissue. Tissue samples from patients will also be archived for future use to replace animal experiments. We also considered using invertebrates including fruit flies and nematode worms, as their use is widespread throughout science for certain applications.

Why were they not suitable?

The detailed structures of interest for us are too different in invertebrate species such as worms and flies, especially the contact sites between nerve and muscles (called neuromuscular junctions) which are of particular interest for the diseases studied by our group.

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?

We have estimated our animal numbers from our previous experience, generating mutant zebrafish models and trialling compounds. Our experience has now shown us that we may need to focus on juveniles up to 14 dpf, which means we have to increase numbers slightly to accommodate working with more over 5 dpf.

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

We reduce animal numbers wherever possible by reviewing our experimental data rapidly following an experiment and planning follow up experiments to resolve outstanding experimental questions. In this way the information generated by our research is maximized while experimental animal use is minimized.

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 the PREPARE and ARRIVE guidelines recommended by the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) for designing our experiments and publishing our results.

Based on our previous work and if needed pilot studies we will minimize the number of fish used in the project. In addition, for lines we are not currently using, we have started to freeze sperm for future in-vitro fertilisation (IVF) if required. This allows us to reduce our animal numbers by approximately 100 animals per line. Previously these lines would have been maintained in the system.

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.

The protocols on this license are designed to minimize any effects on zebrafish after 5 days of development, where the animals have more substantial capacity for suffering. We aim to restrict any harmful effects where at all possible to the early stages before 5 days where we can, closely monitor the health of embryos and humanely kill severely affected individuals before they develop into hatchlings.

In instances where we use fish up to 14 days of age or adults, we will monitor the animals regularly for any abnormal behaviour (hyper/hypoactivity, trouble swimming) or morphological defects (differences in the way they look). If we detect any such abnormalities, which is not due to the modified genetic environment, the experiment will be terminated and we will inform the Named Animal Care and Welfare Officer (NACWO).

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

Muscle, brain, heart and eye are the organs affected by the diseases we are studying; all of these are very complex organs made up of multiple cell types. This means that cell culture models, which generally consist of a single cell type, have limited applicability to patients. Therefore, many pre-clinical studies can realistically only be achieved in whole animals. The detailed structures of interest for us are too different in invertebrate species such as worms and flies, especially the contact sites between nerve and muscles (called neuromuscular junctions) which are of particular interest for the diseases studied by our group.

We previously focused mainly on larvae up to 5 dpf, but our recent work has informed us that working with fish up to 14 dpf will be necessary due to how some of the diseases we study manifest (after rapid development). Despite this, we will still aim to focus our studies, where possible, on larvae 5 dpf or less.

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

In our zebrafish facility, the environmental conditions such as water temperature, food and lighting are strictly monitored according to Home Office guidelines to ensure the health of the animals. All fish are inspected daily and obtain daily live prey (brine shrimp) feeding to allow them to express natural feeding behaviours. Together with the animal technicians in our facility we also test new environmental enrichment options for the fish tanks and use them if they are compatible with routine husbandry and tank cleaning procedures. As zebrafish are social animals, single housing of individual fish during experiments will be kept to an absolute minimum. When the fish are moved to new tanks or put together with other unfamiliar individuals, we will allow sufficient time for them to adapt to the new

environment before starting the experiments. Handling of the animals by using a net is kept to a minimum to avoid unnecessary stress and damage to the animals.

Analgesic treatment (pain killers) is not routinely given after caudal fin biopsy, unless the fish is displaying abnormal behaviour, in which case we would seek advice from the named veterinary surgeon (NVS) about applying analgesics.

Animals will be regularly monitored after caudal fin biopsy by a technician.

As an alternative to fin clipping for genotyping, skin swabbing has been shown to be effective at collecting DNA, while reducing the welfare impact seen with fin clipping. We currently have no practical experience in this technique, however this would be something that we would like to explore and will discuss with the facility NACWO.

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

We will follow PREPARE and ARRIVE guidelines recommended by the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) for designing our experiments and publishing our results.

We will also follow the newest guidelines provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R).

We also follow new research on animal studies, and will use the most efficient and humane methods available.

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

We will always follow the newest guidelines and information provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3R). In addition, online resources including the RSPCA's 'focus on fish' online event (which can be accessed anytime: https://focusonfish.co.uk/) and the zebrafish section of the Norecopa website (https://norecopa.no/species/fish/) also provide valuable information to stay informed about advances in the 3Rs.

In addition, we regularly communicate with the NACWO and other members of the aquatic facility team, who often inform us on best practices working with zebrafish.

We also follow new published research on animal studies, and will use the most efficient and humane methods available.