> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

gradually replaced by new, functional heart tissue. Studying zebrafish has been crucial for identifying the molecular pathways involved in this regeneration process. Previous research suggests that our receptors of interest also play a role in cardiac regeneration. We plan to continue investigating this by using zebrafish with altered gene expression to understand how those receptors influence the different stages of heart regeneration. This will help us uncover the specific cellular functions modulated by these receptors during the regeneration process.

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

This project aims to take what we have learned in the lab and investigate if it can be applied in vivo to study heart and blood vessel disease similar to those seen in humans. We want to find out if changing the expression of certain genes or blocking specific signals can protect against heart and blood vessel diseases. By studying mice and zebrafish, which have similar biological processes to humans, we hope to see if our ideas could eventually help patients.

We are particularly interested in looking at how changing the activity of certain genes and signals affects heart and blood vessel diseases. This could give us clues about new treatments for conditions like heart attacks and heart failure. We will also explore ways to reduce the buildup of artery plaque build-up and help the heart heal better after a heart attack.

If our experiments go well, we hope to publish our findings in a top scientific journal. This could pave the way for new treatments that might help people with heart and vascular diseases in the future.

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

Findings from this work will be made available to other scientists through publication in peer-reviewed journals. We will increase the shared knowledge of the scientific community in the fields of vascular diseases and regenerative biology. Under the previous proju[illegible]

Predicted harms

- Vascular regeneration is investigated following caudal fin amputation, which consist of cutting up to 50% of the fin. Similarly, the fish is under anaesthesia only for a few minutes and resume swimming shortly after being placed back in its tank. The fish might be anaesthetised again to quantify the size of the regenerated tissue but no further surgical intervention will be conducted until the end of the experiment.

- To study vascular repair, we use zebrafish embryos which are briefly anaesthetised to restrict their movement. It is to note, that, contrary to adult fish, the embryos do not rely on gills for breathing as oxygen freely diffuses through their skin. With the discrete prick of a fine needle, we disrupt the vascular network of the embryos. They are then placed back to water without anaesthetics and regain normal activity quickly. We then observe the subsequent repair by observing them under the microscope, for which they must be anaesthetised again, however, no further surgical intervention will be conducted until the end of the experiment.

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

- Few adverse effects (e.g., skin irritation) are expected from the atherosclerosis study (plaque build-up in the arteries) as the mice are not subjected to any surgical intervention. The mouse genetic models we are planning to use are not known to have adverse effects. Atherogenic diets are expected to increase body weight.

- Fish undergoing cardiac cryoinjury will feel only transient moderate to severe pain, which may be managed with the administration of analgesics. The fish resume swimming shortly after the surgical intervention and previous behavioural monitoring indicates that most fish experience only moderate pain. However, it is still to be determined which analgesic agent is best to alleviate the effects of the surgery on the fish.

- Fish undergoing fin amputation will experience mild to moderate pain which may be managed with the administration of analgesic agents. No other detrimental effects have ever been observed in the past or are expected.

- The damage inflicted by the needle to the vascular network of the embryo has little observable detrimental effect. The damaged tissue starts the healing process almost immediately. The embryos are closely monitored, and previous experience showed high survival rate. Nevertheless, it is unknown and currently debated if the zebrafish embryos experience pain at this early stage of development. Repeated anaesthesia at later developmental stages would induce mild distress.

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)?

- Vascular disease/ atherosclerosis study (mouse model): maximum expected severity will be moderate and only attained if unexpected adverse effects occur, such as skin irritation due to the diet. However, most of the animals under this protocol, will be classed under mild severity.

- Heart regeneration study (Adult Zebrafish): 90% of fish under this protocol are expected to experience moderate severity, 10% of fish might experience transient severe pain due to lack of efficient analgesic cover.

- Fin regeneration study (Adult Zebrafish): 90% of fish under this protocol are expected to experience mild severity, 10% of fish might experience transient moderate pain due to lack of efficient analgesic cover.

- Vascular repair study (Zebrafish Embryos): As stated earlier, it is difficult to ascertain pain in this model. However, because of the invasive nature of the injury which will repair over the following period, a maximum severity to be described as moderate is to be expected.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 3 October 2029

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

We have learned a lot from studying cells in dishes about how our growth factors and corresponding receptors of interest work together. Now, we need to check if what we found in those experiments holds true in living organisms. In all the protocols used, alternatives are not available that replicate the response of, for example, the heart or a blood vessel, to injury, or that reproduces the complex cellular environment of a live tissue during repair.

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

If cell culture/co-culture experiments are useful to investigate up to a couple of cell types simultaneously and helpful to dissect and investigate specific signalling pathway by silencing gene expression or chemical inhibition, the build-up of plaque in arteries results from the interaction of several cell types, including the circulatory immune system.

However, when possible, we will use alternative cell culture and ex vivo models as much as possible

signalling mechanisms.

Why were they not suitable?

Cell culture and ex-vivo explants do not recapitulate the complex environment of the biological response to injury. For example, inflammation brought up by immune cells requires a fully functional circulatory system. Additionally, the complex microenvironment made up of extra-cellular matrix and factors secreted by several cell types and their finely orchestrated interactions are only possible in living organisms. Additionally, because we investigate disease progression and regeneration of full organs such as blood vessels or the heart, the time frame of the response is long and relies on a succession of timely orchestrated processes. For example, during zebrafish heart regeneration, all phases, from inflammatory phase (characterised by the primary immune response), the reparative phase (with the activation of the epicardium (the outer layer of the heart) and revascularisation of the heart) and finally the regenerative phase (proliferation of cardiac muscle cells) must occur sequentially to allow successful regeneration. Similarly, in vascular disease, the development of the plaque is dependent on several factors (including blood flow), cells types, and their secreted factors and a

NC3R's EDA unfortunately cannot predict or take into account the animals that are generated via breeding but are not of the correct genotype and therefore that cannot be used. However, if not useful for preliminary studies of my own, I systematically offer the carcasses of those animals to colleagues who need tissues for their own studies. In our institution, communication for sharing resources is efficient and facilitated by the animal technical team.

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

I have over 10 years of experience in breeding animals for research, including mice and zebrafish. I systematically and carefully monitor the number of breeding cages to manage our resources fully. We visit the animal units regularly and perform inventories to understand the breeding performance and monitor the fertility of our experimental animals. Additionally, we genotype our animals to ensure the correct breeding are in place and to minimise the number of animals generated. For zebrafish,

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From experience and informal discussions with colleagues using similar models, the heart injury model is known to be tolerated by adult zebrafish. They experience a transient level of pain which can be managed via the administration of analgesics, so it does not exceed moderate severity; however the best analgesics regiment is yet to be fully defined, therefore a few animals might experience transient severe pain if the analgesia coverage is not fully efficient.

Similarly, from experience, the zebrafish fin amputation model is well tolerated and no adverse effects exceeding mild discomfort have been observed to date with analgesics administration.

Finally, the needle injury model to induce vascular repair in the zebrafish embryo is using the less sentient and more immature life stage. Although debatable if the embryos feel any pain at all, injuries are well tolerated, and embryos continue their development normally whilst repairing the wound. With this new PPL application, we wish to observe the repair process fully which includes the recruitment of immune cells but also the subsequent growth and reorganisation of blood vessels on a longer timeframe.

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are more or less stressful than oral gavage for the mouse.

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

I will consult published guidelines to assist with planning animal research and testing, such as the PREPARE guidelines: http://journals.sagepub.com/doi/full/10.1177/0023677217724823

Other resources are available including guidance and publications from the NC3Rs and Laboratory Animal Science Association.

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

I stay informed on the published literature in animal welfare. In particular, we receive regular updates from our animal technical team and the Animal Welfare and Ethical Review Body (AWERB) about recent advancements in refining in-vivo methods and the 3Rs (Replacement, Reduction and

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