Home Office

NON-TECHNICAL SUMMARY

The genetics and therapy of skin disease

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

No answer provided

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, 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?

To investigate skin and related tissues during health, disease and treatments.

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?

Major advances have been made in the understanding of skin diseases including severe birth mark disorders which are currently untreatable. These disorders affect not only the skin but other organs, and often also carry an increased risk of malignancy. To translate these genetic and biological research findings into clinical practice in humans, we must test our novel therapeutic approaches on appropriate mouse models. By doing this we can investigate whether our therapeutic approaches can be delivered successfully, and whether they are both safe and effective, so that they can be considered for clinical trials.

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

The research is intrinsically translational and focused on generating treatments for untreatable childhood diseases. The final output aim is to identify therapies and/or technologies that will benefit patients directly. There will also be research output in the form of publications, conference presentations and knowledge that will benefit colleagues and other scientists in the field.

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

Short term benefits (from 2020)

Ongoing participation in patient group events will inform the public of our research, engage them with our work and showcase developments into treating skin disease. We anticipate that early aspects of our research will be published in the coming two years and presented at conferences.

Medium term benefits (from 2023)

Publications and presentations will inform others of our work, assisting and advancing technologies, and forming the basis of further grant applications.

Long term benefits (from 2025)

Assuming successful outcomes, our preclinical research on mouse models will be translated into clinical trials on patients from around 2025. Longer term benefits would be extrapolation of this work to other disorders.

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

This project is already the product of several key collaborations, particularly as regards the delivery systems. Results of the current projects will automatically be shared with the collaborators, allowing them to expand knowledge of their systems and share that knowledge within their respective fields.

Knowledge will therefore be disseminated by our team throughout clinical genetics, dermatology and oncology circles within the medical arena, human molecular genetics and genetic therapy circles in the scientific arena, and delivery systems arenas via our collaborators.

Publication of results will be a priority, as well as dissemination to stakeholder groups in person and using social media and mainstream media outlets. Where practical and possible we will publish unsuccessful approaches

Species and numbers of animals expected to be used

- Mice: 521

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.

We are using the mice as a model of skin disease because mouse models are available that have the same genetic mutation that humans with the disease have. This is ideal for progressing with our preclinical investigations of possible therapeutic agents as they will provide a platform to move forward with our research to a practical in vivo system (mice with skin disease causing genetic mutations). Therapeutic interventions on humans with skin disease might in the future be carried out on any postnatal stage. Therefore, all postnatal stages in the mice are of interest to our studies as they will model both early and later aspects of skin disease. In order to best model skin disease in mice, we may also cross any of these strains with other strains with genetic mutations associated with the disease which is not expected (from clinical observation in humans) to have a phenotype at all on its own, but may exacerbate any of the phenotypic features in the strains described here.

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

Mice with skin disease causing genetic mutations will be generated using standard breeding methods. Baseline phenotyping will involve recording the mouse's phenotypic features and basic non-invasive imaging methods (e.g. dermatoscopy). Where it will be of value, imaging methods requiring general anaesthesia may be carried out and skin or blood samples may be collected for analysis to see if the treatments have worked. Administration of substance will involve application of the substance, most likely to the ear but could be to any part of the body. The application procedure may involve preparation of the skin (e.g shaving) followed by treatment with the topical cream or ointment. To ensure that the substance remains on the skin for an appropriate amount of time it may be necessary to house the mouse in a single cage for a period after the treatment to prevent other mice grooming away the

substance. The mice will also be phenotyped after substance administration in order to investigate the efficacy of the substance for treating the phenotype. All experiments will end with culling of the mice using a schedule 1 procedure.

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

Our experiments havee been designed to minimise adverse effects on the mice. Phenotyping and sample taking may cause mild, transient pain or distress but this should last no longer than a few seconds. In the majority of imaging procedures, the mice will be under general anaesthetic to minimise distress. Substance administration can potentially cause minor skin irritation, but efforts are made to select candidate substances for their lack of toxicity. On an occasion where the skin is sore as a result of substance administration, local anaesthetic will be used to minimise suffering. As we will be observing the mice regularly following substance administration the mice will only suffer from mild irritation for a short period of time before it is detected. We intend to cull mice before any potential melanoma metastases develop. However, undetected disease may cause pain, weight loss, metastatic tumours and abnormal behaviours. We estimate that this will affect less than 5% of our animals and mice will be culled at the point in which the phenotype is detected.

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)?

Mild (expected of phenotyping / substance administration) = 90%

Moderate (unexpected) < 10%*

*Our goal is to achieve 0% moderate severity. However, we believe due to the nature of our mouse models and investigative nature of substance administration there is a small chance that some mice may experience moderate severity.

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?

A mouse model with the precise genetic mutation that causes a skin disease that we are interested in treating in humans is available. This provides an excellent opportunity to study and treat the disease in

vivo. Data from these studies will inform us on the effects of the drugs in a living system, with functioning networks (e.g. vasculature, nervous system etc.).

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

We already and will use in vitro approaches in parallel to our mouse research to inform our experiments. These include experiments on human patient-derived biopsies and cell lines.

Why were they not suitable?

While non-animal alternatives can provide useful data, they limited in that they do not model the complete skin in vivo which is connected to the nervous system, immune system, vascularised, under the influence of endocrinological factors and systemic signalling molecules.

There is often limited availability of patient tissue which prevents the design of experiments as there is little to no knowledge of what will be available for study until the moment of surgery. Even when it is available, it is often variable in nature, from different parts of the body from different patients. This provides variable data with limited opportunity to carry out a carefully controlled, designed study.

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?

In order to estimate the number of animals we will use we have carried out power calculations using an online tool. Estimations are listed below. Where possible, these estimations are carried out using what we can learn from published data. In cases where there is not yet published data we will need to carry out pilot studies with a limited number of mice to determine the distribution and difference of data points in two conditions in order to more accurately estimate the number of mice required for an experiment.

Estimation of number of mice required for breeding pairs

This depends on the length of time between experiments, the success of breeding and the numbers they generate. Therefore, assuming we were required to have 10 mice available for breeding at any given time, retiring them every 6 months to avoid detrimental phenotypes. We would use 20 each year. Over the 5 years of the project this would be 100 mice. At any given time we may need to breed both strain 1 and strain 2 mice. Therefore, for the 2 mouse lines we are estimating the maximum number of mice we will need for breeding is 200 (see table 2)

Estimation of sample size for studies on strain 1 mice

a) The primary outcome measure will be a decrease in pigmentation

b) The statistical test is a paired one-sided students t-test, with 95% significance at 80% power

c) The effect size considered significant will be a 20% decrease in pigmentation for experiments designed to achieve successful treatment of the phenotype. We account for 10% variability in the results of these experiments between mice. However, until we do preliminary experiments we cannot accurately estimate this.

Using this data with an online calculator the sample size is 8 mice in each group, with two delivery approaches to be tested, with two different concentrations of genetic material. Therefore, the final sample size is 32 mice per experiment. For two experiments and accounting for a 10% attrition rate we estimate we will use 71 mice in protocol 4 (see table 2).

Some mice will be used for baseline characterisation for preliminary studies to establish baseline measurements to direct the therapeutic interventions experiments. We estimate we will require 10 mice in protocol 2 (see table 2)

Estimation of sample size for studies on strain 1 mice

a) The primary outcome measure is the number of melanocytic lesions over the first 6 months

b) The statistical test is a paired two-sided students t-test, with 95% significance at 80% power

c) The effect size considered significant will be a 10% difference in mean number of melanocytic lesions with the baseline estimated to be 3 per mouse, with a variability of 20%

Using this data with an online calculator the sample size is 31 mice in each group, with two variants to be tested, plus a wild-type control group – final sample size is 93 mice in either protocol 2 or protocol 3 (see table 2)

Estimation of sample size for studies on strain 2 mice

a) The primary outcome measure will be a change in pigmentation (also melanoma incidence will be closely monitored).

b) The statistical test is a paired one-sided students t-test, with 95% significance at 80% power

c) The effect size considered significant will be a 20% change in pigmentation for experiments designed to achieve successful treatment of the phenotype. We account for 10% variability in the results of these experiments between mice. However, until we do preliminary experiments we cannot accurately estimate this.

Using this data with an online calculator the sample size is 8 mice in each group, with one control group and two experimental groups, the final sample size is 24 mice. Accounting for a 10% attrition rate we estimate we will use 27 mice in protocol 2 or 3 (see table 2).

Table 2: Estimation of number of mice that will be used

	Protocol 1	Protocol 2	Protocol 3	Protocol 4

Strain 1	100	28	18*	71
Strain 2	100	102	102*	
Total (521)	200	130	120	71

*Protocol 3 will only be used if protocol 2 does not achieve experiment goals

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

Experiments follow a repeated measures design whereby mice will be treated and phenotyped repeatedly. This will allow us to monitor the effects of substance administration over multiple time points while avoiding the use of multiple groups of mice to carry out these experiments.

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

To optimise the number of mice in our studies, pilot investigations have been and will be carried out on cell in vitro and skin biopsies ex vivo to determine which approaches are most promising and which are most likely to deliver a successful outcome. This will minimise the number of mice that are needed as fewer investigative experiments will be carried out on the mice whereby little is known about the potential outcome of the therapy being tested.

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 animal models that we will use in the project are mice with genetic mutations / phenotypes which model skin disease. These models present with a phenotype which does not cause an aversive effect in the mice. In certain circumstances there will be an increased risk of melanoma but this can be managed by observation and culling of the mice before they reach a stage that causes pain or suffering. In particular, where a carcinogenic substance is used to induce melanoma at a particular location on the skin we are able to monitor this precise location to make sure procedures are carried out before the melanoma causes any aversive effects to the animal.

Phenotyping procedures which may cause distress to the animal because of restraint will be done under general anaesthetic. This will minimise the stress to the animal while allowing us to collect the data as quickly and efficiently as possible. In certain circumstances such as ear biopsies, the temporary

restraint and minor transient pain may mean it is less intrusive and stressful to perform the procedure without anaesthesia, or with local anaesthesia instead.

Substance that will be administered to treat the phenotype will be selected for their reported or predicted lower toxicity. This is because the goal of our experiments is ultimately to treat patients and very toxic drugs would be unsuitable. In the event of an adverse reaction the mouse will be treated if this is possible (e.g. local anaesthetic) or culled to prevent worsening of the condition.

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

We cannot use animals that are less sentient than mice because:

a) Mouse embryos are not appropriate/accessible for topical application of substances

b) Procedures cannot be solely performed on terminally anaesthetised animals because the effect of the therapy needs to be monitored for some time after application.

c) In general, previous therapies for melanocytic disease including melanoma have been tested on mice before being translated into humans, with mice being considered the least sentient animal model close enough physiologically to humans for this disease. Our efficacy and safety data will therefore be comparable to previous work.

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

Staff will carry out procedures in an accurate and efficient manner. Given the nature of dermatology research, mice will be monitored for surface abnormalities which will be important for our results and carrying out experiments effectively. This will minimise harms to the animal by identifying phenotypes early that may eventually be harmful to the mouse.

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

Reference to the topic specific resources on the NC3Rs website will be used to ensure experiments are conducted in the most refined way. This resource provides guidelines and resources on anaesthesia, analgesia, euthanasia, handling and restraint, humane endpoints, microsampling and welfare assessment which will be relevant for our project. Experiments will be planned according to PREPARE guidance. Experiments will be conducted with care taken to design and collect information that satisfies ARRIVE (Animal Research: Reporting of In vivo Experiments) guidelines.

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

By subscribing to the NC3Rs newsletter and discussing advances that could be implemented in our research with my research team. Additionally, we will receive communication from named persons and complete annual refresher training.