Home Office

NON-TECHNICAL SUMMARY

Targeting parasitic helminths with drugs

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
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

parasites, drug discovery, therapy

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

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 identify and develop novel chemical substances which get rid of parasitic worm infections effectively, and which have properties which would allow them to be developed into medicines. The project will also assess the ability of existing drugs, developed for other purposes, to act against parasites. To aid these aims we will also try to increase our understanding of how drugs are taken up into the parasite and how they work.

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?

Gut dwelling parasitic worms are extraordinarily common, impacting on the health and wellbeing of around one quarter of the world’s population. In addition, parasitic worms impact on the welfare of wildlife and domestic animal productivity. Drugs which are currently used to treat these types of parasites are losing their effectiveness, therefore, new drugs are urgently needed.

Thus, this project aims to discover new drugs and assess the possibility of repurposing existing drugs as anti-parasite therapies.

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

The primary output of this project is to identify novel or repurposed compounds which target parasitic worms *in vivo*. To support this output we expect to also identify the route by which anti-parasitic compounds are taken up into parasite tissues, as well as an understanding of how they kill the parasite (their mechanism of action). This knowledge is important as it will help to strategically develop other drugs, that target these mechanisms and uptake routes.

We intend to share our findings with the scientific community and will publish at least three new papers describing novel compounds, mechanisms of action and/or of uptake. In addition, we expect to present our work, in the form of a poster or a talk, at both national and international conferences.

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

In the short-term, we will publish our work and discuss our data at scientific conferences. This will help the sharing of our compounds with other researchers, who will be able to test them against other types of infection. Moreover, we have established collaborations with other groups also interested in neglected tropical diseases, and have utilised these networks to test our compounds against other infections.

Our long-term goals is for new drugs which effectively kill parasites, to significantly help in controlling parasitic worm infections in developing countries. Within 5-10 years it is highly likely that we will have discovered and optimised, through chemical modification, new anti-parasitic drugs suitable for clinical trials in man and/or use in veterinary applications.

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

Sharing our data sets, where appropriate, at conferences and invited talks will allow researchers to test our compounds against other types of infection. We have already embraced this approach and have published the efficacy of some of our existing novel compounds against worms we ourselves do not study.

In addition to publishing positive outcomes of our research we also strive to publish compounds which have failed to eliminate worms *in vivo* as this knowledge is useful when trying to understanding how drug structure relates to effectiveness *in vivo*.

We also strive to publish our methodologies and workflows allowing others to adopt our working patterns if appropriate.

Species and numbers of animals expected to be used

- Mice: 3750

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 studies focus on the infection of adult laboratory mice with the gut dwelling parasitic whipworm *Trichuris muris* (*T. muris*). *T. muris* is the equivalent mouse parasite of *Trichuris trichiura* (*T. trichiura*), the whipworm which infects humans. *T. trichiura* and *T. muris* are virtually identical and therefore the mouse model enables us to develop new therapies to treat *T. trichiura* in humans.

Any chemical substances which we test *in vivo* (in mice) will already have been shown to successfully target the parasite *in vitro*. However, testing cannot be solely performed *in vitro* as the activity of any substance *in vivo* may differ to the activity seen in a petri dish *in vitro*.

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

Mice will be treated with chemical substances orally (no more than twice a day for five days) or by injection into the vein (no more once daily for 5 days) or by injection in to the abdominal cavity (no more than once a day for five days) or by injection under the skin (no more than twice a day for five days). Some mice will have received a parasitic infection up to 5 weeks before treatment with the

chemical substances. Parasites will be given via the mouth. During the chemical substance treatment some mice will have small volumes of blood withdrawn for their tail veins, with no more than two samples taken in any 24 hours period and never exceeding the published guidelines on blood sampling.

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

Administration of parasites via the mouth will causes short term discomfort and stress from the restraint. This will occur at each administration but will resolve once the procedure has been completed, which typically takes less than ten seconds to complete.

Oral dosing of substances may enter the airways or damage the tube leading down to the stomach. This will occur rarely (<0.1%) and can be avoided by correct holding of the animal and good technique. If the airways are damaged we would expect to see an immediate increase in breathing rate, fur standing on end and a hunched posture. Such animals would be humanely killed.

Parasite infections can cause dehydration due to diarrhoea. This will occur rarely (<0.1%) and only when the later and larger larval stages and adult worms have developed in the gut. It can be managed by providing wet food ("mash") or fluid therapy and usually animals respond within 24 hours. In the rare occasion that an animal doesn't improve after one day of receiving fluid it would be killed humanely.

Giving a mouse a novel chemical substance may make the animal poorly, typically losing body weight, being less active and adopting a hunched posture. We very carefully assess our chemical substances, for example by analysing their ability to damage or kill cells (cytotoxicity) *in vitro*, prior to treating any mice *in vivo* and thus these effects are rare (1-5% for tolerability testing and <1% in efficacy assessments). Careful daily monitoring of the animals immediately following dosing ensures we do not go above unacceptable harm as defined in our licence. Weight loss should plateau and begin to recover one day after the cessation of drug treatment.

Taking blood from the tail vein may cause local bruising and blood collecting outside of the blood vessels. This is rare (<0.1%) and associated with moderate discomfort. Monitoring of any mice experiencing bruising daily following blood sampling is important with bruising usually resolving within 3 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)?

The procedures employed under this licence are classified as moderate. There are two main possible harmful effects, the first relating to administration of the novel chemical substances and the second to removing of blood samples. Both are expected to be rare events (1- 5% and <0.1% respectively).

For tolerability testing mice will be treated with novel chemical substances orally, or by an injection into the abdominal cavity or into the bloodstream, or under the skin. As these are novel chemical substances it is possible that, occasionally, the mice will react badly to these chemical substances. We expect these to be infrequent events (experience to date suggests between 1-5 %) as any chemical

substances administered to mice will have gone through rigorous testing *in vitro* to show that they do not kill cells. Mice will be checked daily after treatment with the novel chemical substance. We will look for signs that the animal is beginning to suffer and any mouse affected will be humanely killed.

Efficacy assessments: Chemical substances which are well tolerated by mice in tolerability studies will then be assessed in infected animals. This will enable us to establish how long the chemical substances last in the bloodstream and the ability of the chemical substances to eliminate the parasite from the mouse. Having tested these chemical substances in uninfected mice, we expect adverse effects due to giving animals the chemical substances to be rare (<1%). In order to to test the effectiveness of our chemical substances against the parasites, mice will be infected with parasites using standard procedures, chemical substances administered and blood repeatedly withdrawn from a tail vein. The main risk of harm to the mouse in these studies is excessive blood loss. We expect loss of too much blood to be very rare (<0.1%) as we will carefully control the frequency and size of the blood sample taken. We will ensure bleeding has stopped after each sampling. If the bleeding cannot be controlled, resulting in a blood loss greater than defined limits, the mouse will be humanely killed.

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?

Our studies are interested in identifying novel chemical substances which act against the gut dwelling parasitic whipworm *Trichuris*, utilising the mouse version of the human parasite, *T. muris*. *T. muris* in the mouse is a good model of *T. trichiura* infection in humans. Importantly, the mouse species of *Trichuris* is remarkably similar to the human species of in terms of its genes, molecules and interactions with its host. Thus the mouse model of the human disease enables us to develop new therapies to treat *T. trichiura* in humans.

In order to investigate novel chemical substances, which work against *Trichuris*, we need to grow the parasite *in vivo* as, despite work attempting to generate *Trichuris* outside of animals, *in vitro* the adult stages do not develop. Moreover, to determine if chemical substances have the potential to work in humans, they need to be tested *in vivo* as there are many additional factors, for example, how long the chemical substances last in the host, how available they are and how quickly they degrade, which cannot be effectively modelled *in vitro*.

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

In vitro screening against *T. muris*: The aim of the project is to discover novel ways to treat gut dwelling worm infections, through the discovery of new drugs and the use of existing drugs which haven't been

used against worms before ("repurposing"). *In vitro* screening assays allow us to examine particular properties of the chemical substances, for example, checking how good they are at killing the worm. This *in vitro* screening provides essential data on the effectiveness of chemical substances directly on the parasitic worms and will always precede any use of substances *in vivo*. Thus, using our automated high throughput *in vitro* screening assay, hundreds of novel chemical substances or drugs for repurposing will be screened against the adult stage of the parasite for anti-parasitic activity. However, this still requires mice to generate the adult parasite.

In vitro screening against *T. muris* eggs: We will also screen any novel chemical substances, capable of killing adult *Trichuris* worms, *in vitro* against early larval stages of the parasite via our *in vitro* egg hatching assay.

In vitro cytotoxicity screening: Chemical substances will be screened for any toxic activity against mammalian cell lines prior to any *in vivo* treatment in mice, to eliminate chemical substances which have a directly toxic effect on mammalian cells.

Substances may also be screened utilising the free-living non-parasitic nematode *Caenorhabditis elegans* (*C. elegans*) which can be used as a model for parasitic nemtodes in some instances.

In vitro pharmacokinetics (PK) analyses: in order to inform the fate of a chemical substances *in vivo* (its pharmacokinetics properties), substances which show good anti-parasitic activity will be screened *in vitro* to determine PK parameters e.g. half life and absorption.

Why were they not suitable?

In vitro screening against T. muris: whilst an important step to identify substances with anti-parasitic activity we know that anti-parasitic activity *in vitro* does not always translate to anti-parasitic activity *in vivo*. Thus, following *in vitro* screening, drug candidates need testing in mammalian systems to explore the effectiveness of the drugs in eliminating the parasites from their host.

In vitro cytotoxicity screening: whilst a good indicator of possible toxicity, the ability of an animal to tolerate a novel substance can ultimately only be established in a fully intact organism.

Screening utilising the free living model nematode *C. elegans*: we did consider screening utilising *C. elegans,* however, previous work in which we have screened against both *T. muris* and *C. elegans* has highlighted that efficacy across the two different nematodes does not always correlate. Therefore, this could lead to false positive and negatives.

Screening utilising larval stages of *T. muris*: similarly to *C. elegans*, we considered screening against the L1 larval stage of the *T. muris* parasite, as these can be generated *in vitro* from parasite egg stocks, without the infection of mice. However, published data demonstrates that chemical entities which are efficacious against the L1 stage do not always correlate with those that are effective against the adult stage. In addition, although targeting larval stages of the parasite could reduce the number of worms which reach adulthood, this is unlikely to treat the morbidity associated with *T. trichiura* infections which is due to the presence of adult parasites.

In vitro pharmacokinetic (PK) analyses: Whilst the *in vitro* assays can aid in prioritising substances which are likely to have a good profile, determining the full potential of the novel chemical substances

requires studies in the animal to understand the fate of a substance. This includes measuring concentrations of the substance in the blood over time in order to understand how it is metabolised *in vivo*. These data are important and cannot be obtained in any other way.

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 mice numbers projected have come from the sum of:

In vitro worm generation: from historical data we know the number of worms we anticipate to generate per mouse, and the number of replicates we need for robust data from our *ex vivo* screening platform. We can utilise this data to determine the number of mice required for generating worms for *ex vivo* screening.

Tolerability studies: the numbers of mice used in these studies have been projected based on the number of concentrations of chemical substances we test, multiplied by the number of substances we estimate to progress to testing *in vivo* to see how well a mouse tolerates the substance.

In vivo efficacy studies: these estimations have been based on the known numbers of mice required for each treatment group from historical studies which have allowed us to detect significant differences, combined with an estimation of the number of treatment groups we will test.

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

In vivo efficacy experiments have been designed utilising the NC3Rs Experimental Design Assistant as well as with consultation with an external statistical consultant.

Egg doses for *in vitro* worm generation has been carefully monitored over several years to optimise the numbers of healthy worms generated whilst minimising any adverse effects from a high worm burden.

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

- Repurposed drugs: where possible we will use existing data on *in vivo* tolerability available for our analyses of repurposed existing drugs thus avoiding unnecessary animal usage for tolerability studies.

- We have revised our experimental design such that we no longer always include our "gold standard" drug treatment, which routinely results in complete worm clearance, as a positive control.

- We have carefully considered group sizes and experimental design, where possible, to test several chemical substances in one experiment to minimise the number of vehicle-treated control animals required.

- We routinely take samples for pharmacokinetics analysis for all our treatments. These samples are archived at -80C and thus if any substance is of interest we do not need to repeat the initial experiments in order to gain data about how the substance behaves *in vivo*.

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.

Choice of animal model

Our studies focus on the use of the laboratory mouse. *T. muris* in the mouse is a validated model of *T. trichiura* infection in humans. Importantly the mouse species of *Trichuris* is remarkably similar to the human species of *Trichuris* in terms of its genes, molecules that stimulate the immune response and the way it interacts with its host. Thus the mouse model of human trichuriasis enables us to develop new therapies to treat *T. trichiura* in humans.

We use both wild type and severe combined immunodeficiency (SCID) mice. SCID mice lack part of their defence system (their immune system) that would normally get rid of parasites. As such, they provide a robust way to generate *Trichuris* worms without the need to repeatedly inject mice with an immunosuppressant. Importantly, if kept in a very clean environment, SCID mice are healthy.

The protocols employed are well established in our lab and designed not to induce suffering in animals.

T. muris is a natural parasite of mice and is well tolerated with no adverse effects. In order to assess how effective a drug is against the parasite (drug efficacy) we will usually infect mice with low infection doses (eg 40-50 eggs) to establish the numbers of worms typically found in wild mice and humans.

Novel chemical substances will only be delivered *in vivo* after careful *in vitro* analyses to exclude cytotoxic effects. Initial *in vivo* studies (tolerability studies) will be in the absence of infection with each mouse treated one at a time and observed for any adverse effects greater than short term distress (e.g. change in breathing pattern or mobility, poor grooming) prior to treatment of further mice.

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

T. muris takes 35 days to develop to the adult stage of the parasite and therefore infections cannot be performed under terminal anaesthesia or at an immature lifestage.

We did consider screening our substances for anti-worm activity using the free-living model worm *C. elegans* or the L1 larval form of *T. muris* (which can be hatched from pre-existing egg stocks), however, previous work in which we or others have screened against both *T. muris* adults, *T. muris* L1 and *C. elegans* has highlighted that efficacy across the two different nematodes and the two different lifecycle stages does not always agree. Therefore, this could lead to false positive and negatives.

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

- We routinely perform *T. muris* infections with no side effects other than minor discomfort following scruffing / oral gavage which is very transient.

- Throughout treatment with novel chemical substances we will ensure the mice are well monitored with bodyweight, grimace score, body condition and general observations recorded following treatment to ensure no adverse effects are experienced.

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

We will follow the ARRIVE guidelines for all our experimental work. In addition, prior to any animal studies we will prepare and submit a full experimental study plan to the animal unit to ensure all studies are carried out in line with best practices. We will conform to the principles described in the Working Party report "Refining procedures for the administration of substances" in Laboratory Animals (2001) 35, 1-41.

Use of male and female mice: when growing adult stage parasites *in vivo* we prefer to use male mice as they tolerate the infection better than females; equally in our initial assessment of how well the mice tolerate the drug and clear the worms we prefer to use male mice as they are larger and less likely to experience any harm. However if substances show anti-worm activity in these pilot studies it is important that we then test our novel candidates in females where the way the substances behave inside the mouse may differ and thus alter our assessment of how translatable our work will be into humans. To minimise harm to females used we will only use mice above 22g weight.

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

We will stay informed of 3Rs advances through

- NC3Rs newsletters

- animal unit newsletters

- discussions with other *in vivo* researchers

- seminars put on through the animal unit

Any changes to best practice will be discussed with the NACWO and implemented where appropriate.