Home Office

NON-TECHNICAL SUMMARY

Development of novel therapeutics for pain

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

Pain relief, Inflammatory pain, Neuropathic pain

Animal types	Life stages
Mice	adult
Rats	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?

The purpose of this project licence is to increase our knowledge and understanding of the mechanisms involved in the pain pathway and to use that knowledge to identify and test new candidate molecules in relevant models. This will ultimately help us develop new potential treatments for pain.

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?

Pain is defined as ‘an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage’, and is represented by various conditions such as low back pain, neuropathies (where the pain is due to damage to nerves), and arthritis (joint pain). Chronic pain has been reported to be one of the most prominent causes of disability worldwide.

The treatment of both inflammatory pain (in which the pain is caused by tissue damage) and neuropathic pain is currently poor and is considered a major area of unmet need. Despite drugs being available for the treatment of these conditions, less than half of all patients achieve adequate pain relief from current medications. Existing therapies also have major side effects (sedation, nausea, vomiting) which can limit their effectiveness, and discourages patient compliance. Physicians generally agree that even small improvements in safety and/or tolerability would be good progress. Any novel therapies would be expected to have better profiles than existing treatments.

Our team has been focused on developing novel treatment options for a variety of painful conditions with a few compounds developed under previous similar licences being trialled in the clinic currently. We perform a large amount of in vitro (using cells in the laboratory) experiments to understand the mechanisms of pain, develop efficient compounds and test the efficacy of these compounds in relevant disease animal models to give us an extra layer of confidence in developing these new medications. Through continuing this work, we intend to develop analgesics (pain medicines) which are more efficacious and safer than the current medications.

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

New candidate drugs/compounds (those that are being developed and trialled but have not been approved for use yet) for inflammatory and neuropathic pain and an increased understanding of the mechanisms responsible for these painful conditions will be the main outcomes of this project licence.

By testing the effects of novel compounds on either the development or treatment of pain, we hope to identify and develop new therapies for painful conditions such as arthritis and neuropathies. Tissue collection during the studies will increase our understanding of the mechanisms involved in painful

conditions and validate our targets. This may also help with the development of biomarkers, which are currently lacking for a majority of analgesics used clinically.

Additionally, findings will be disseminated in the form of papers or presentations at conferences to help advance the field. Some of the work may also contribute towards patents for new compounds. Any unsuccessful approaches may be published in open access platforms, as long as it does not contain confidential data.

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

The work being undertaken under the realms of this licence will be of benefit to the wider scientific and medical community, and eventually patients. The primary potential benefit from work carried out under this licence is the development of new and better treatments for painful conditions. Any new treatments which come from this work would be expected to show better efficacy and tolerability profiles (resulting in lesser side effects) than existing treatments.

The short-term benefit of the work undertaken will be progression of pain projects through the various stages of drug discovery, starting from target validation to the development of a candidate drug to conduct clinical trials.

Most benefits arising from the work being undertaken will be observed in the long term (10-15 years) due to the time scale required to develop new medicines. For example, compounds that were tested under the forerunners of this license in the past decade are now in Phase 1-2 clinical trials for chronic pain.

It is well known that multiple diverse mechanisms are involved in pain pathways leading to a complex pathology, which is still largely less understood. Except for the gabapentinoids class (analgesic to treat neuropathic pain), there has been little development in new pain medicines over the past decade. Recently, the development of targeted therapies such as inhibitors of nerve growth factor (NGF, which is an important mediator of nerve growth), have shown remarkable efficacy in clinical trials. Despite being challenged by safety issues this approach has helped set in motion a revolutionary change in the treatment of chronic pain.

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

Our team works in collaboration with many different academic and pharmaceutical institutes to help with the progression of the field. Data will be presented where appropriate and where internal confidentiality permits, at both national and international scientific meetings and conferences. We also expect to publish results, not subject to confidentiality, in peer-reviewed journals for the benefit of the wider scientific community. Where possible, we will publish results on platforms that have open access.

Species and numbers of animals expected to be used

- Mice: 7000
- Rats: 750

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.

While most of the studies carried out under this licence will be using mice, a few studies must be undertaken using rats. Testing in rats will allow us to provide information, for example, efficacious doses which can be used in future studies such as those assessing the safety of the drug, which are required for regulatory approval following the selection of candidate drugs. There is wide literature supporting the use of mice and rats in models of pain, which will be used to provide a background to the studies prior to instigating any work. We may also use genetically modified mice in all procedures, which will enable us to better understand pain pathways and provide information about the selectivity of test substances. All studies will be performed using adult rodents as the models we are using are representative of clinical conditions such as arthritis and neuropathic pain that commonly occur in adults. These models have been well established for decades, while also undergoing refinement when new information about human disease pathology or animal models is available.

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

Any animal arriving in our animal unit will be allowed to acclimate in their home cages for 7 days, with food and water provided ad libitum and general daily checks being performed. Most of our studies will involve animals being microchipped for identification and blinding purposes, following which they will be allowed to recover before carrying out any procedure.

Inflammatory pain models: The typical animal will undergo dosing with substances causing inflammation into the foot, ankle or knee joint, following which the animals will be evaluated for the desired behavioural endpoints at various times post-dose. Behavioural measurements may range in time from a period of a few minutes in the acute models, through to 4-5 weeks in the case of the more chronic joint pain models. Once a pain-like behaviour is induced, the animal will be dosed with compounds that are being developed to treat pain, to investigate if they can reverse the pain behaviour in the animal. If a compound is being developed to prevent pain, it may be dosed before or alongside the inflammatory substance. During or at the end of the experiment it is anticipated that samples (such as tissues or blood) may be taken for subsequent analysis such as biomarkers (a substance found in blood or other tissues that can indicate the presence of a disease) and pharmacokinetics (how the body interacts with the administration of the compound). In the acute models of inflammatory pain where interventions may be performed as early as 30 minutes after foot injection of the inflammatory substance, using an analgesic will interfere with the scientific outcomes and hence will not be employed. However, in the chronic models involving the ankle or knee joint, no behavioural testing will be performed in the first 3 days after dosing of the inflammatory substance. We will hence employ local analgesia to help with recovery if no interventions such as tissue collection is required in the immediate 48 hours after dosing of the inflammatory agent. However, we will consider running pilot studies every time a new target is being investigated to determine the effect of local analgesia on tissue collection

and analysis to determine any biological effects. If none are observed, we may consider using local analgesia.

Neuropathic pain models: The typical animal will undergo nerve ligation surgery (where the sciatic nerve will be ligated using a suture) under general anaesthesia or the administration of a chemotherapeutic agent (anti-cancer drugs, administered into the blood vein or abdomen commonly), following which the animal will be evaluated for the desired behavioural endpoints at various times post-surgery or dosing, which may last until 4-6 weeks. In the surgical model, the animals will be allowed to recover for 3 days before any behavioural testing is performed. As the chemotherapy model will involve multiple injections of the chemotherapy and this is not expected to cause any distress to the animal, they may be tested at any point in the study. Once a pain-like behaviour is induced, the animal will be dosed with compounds that are being developed to treat pain, to investigate if they can reverse the pain behaviour in the animal. If a compound is being developed to prevent pain, it may be dosed before or along with the inflammatory substance. During or at the end of the experiment it is anticipated that samples may be taken for subsequent analysis such as biomarkers and pharmacokinetics. In the surgical nerve ligation model, we will employ peri-operative analgesia to ensure smooth recovery from the surgical procedure. The animal will be allowed to recover for 3 days before any behavioural testing is performed. However, if any interventions such as tissue collection is planned within the 48 hours after surgery, no analgesics will be utilised as it may interfere with the study outcomes. However, we will consider running pilot studies every time a new target is being investigated to determine the effect of peri-operative or local analgesia on tissue collection and analysis to determine any biological effects. If none are observed, we may consider using them. If in any instance, an unexpected drug interaction with any novel compound is observed, the peri-operative analgesia regime will be reviewed to identify alternatives to avoid this in further studies. No analgesia will be employed in the chemotherapy induced peripheral neuropathy model, as such measures are not available in the clinic and the animal may be tested any time after administration of the chemotherapy.

All behavioural tests employed are used to either measure a painful response to a mechanical or heat/cold stimulus. Specified cut off points are used to avoid adverse effects of testing. These tests are animal equivalents of the measures that are used to assess pain in humans. All models mentioned have been refined to cause the least suffering. From our experience, the animals are generally active without any signs of distress, apart from those expected due to the nature of the model.

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

Inflammatory pain models: Due to the nature and administration of compounds, the common adverse effects that may occur are redness, swelling and mild discomfort at the site of administration. The injection itself and administration of control substances are not expected to cause any harm, apart from mild and transient discomfort. The animals are expected to develop a pain-like behaviour in the form of mechanical and thermal hypersensitivities (reduced threshold to feel pain, pressure, heat and cold). However, from previous experience, this does not affect the normal behaviour of animals.

Neuropathic pain models: From previous experience, animals are expected to show an uneventful recovery from the nerve ligation surgery, similar to that seen in humans undergoing surgery under general anaesthesia. Problems with wound breakdown caused by animals licking the site of surgery and lameness in the affected limbs are most commonly seen over the first few days. Following this,

except for mild gait changes, very few adverse effects are ever seen such as wound opening. However, these do not have any effect on the overall welfare of the animal. The animals are expected to develop a pain phenotype in the form of mechanical and thermal hypersensitivities. In the case of administration of a chemotherapeutic agent, the animals may be expected to show signs of transient discomfort and pain-like behaviour such as mechanical and thermal hypersensitivities. Additionally, some swelling and redness at the site of chemotherapy administration may last for a few days. No effect on the normal behaviour of the animals is expected.

Unless otherwise specified, the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes and frequencies resulting in no more than transient discomfort and no lasting harm. These tests are not expected to produce any adverse events. The behavioural endpoints used to measure mechanical and thermal hypersensitivities are not expected to have adverse effects. To further refine the process, we have specified cut off points to prevent any injury or tissue damage due to testing.

The adverse effects mentioned above are all expected to be transient and resolve over a few days without affecting the well being of the animal. If any adverse effects beyond that expected occurs, the NVS will be consulted depending on the nature of the adverse effect. Any severe adverse effects resulting in an alteration of appearance, food and water intake or natural behaviour will result in the animal being 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 – 10%

Moderate – 90%

Rats:

Mild – 10%

Moderate – 90%

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?

The use of animal models of acute and chronic pain conditions is fundamental to providing insights into molecular, cellular, and systems organisation of pain that can otherwise be hard to determine clinically. These animal models have been designed to capture either some or most of the characteristics we see in the clinic with patients suffering from various painful conditions. Such animal models offer the ability to determine the analgesic efficacy of novel compounds.

Non-animal alternatives such as in-vitro testing of neurons have been extensively studied by many groups and show that a proportion of the fundamental properties are lost in culture, which leads to misinterpretation of results. For example, in vitro cell culture systems have limited utility in informing us about pain perception, which can only be determined by behavioural studies. Often, retaining one of the fundamental properties is insufficient to inform us about the prevention of pain due to the complexity of the systems involved in the pain pathway. In addition, testing in culture systems alone cannot provide information about the efficacy of drugs or the severity of pain, which is crucial to model and determine doses that are to be assessed clinically. The other non-animal alternatives such as organ-on-chip technologies to study arthritis have been gaining traction. Although they do a particularly good job of being able to study interactions between different cells/tissues, they are unable to fully recreate the entire joint environment, which may lead to false results, and ultimately the development of either an unsafe or ineffective compound for human use.

As a result, we carefully perform and design these animal experiments to obtain valuable information about translation in the clinic, which cannot be achieved by non-animal alternatives. Where possible, extensive in-vitro experiments are performed to understand more about the target and characterise responses produced by compounds, to help choose the best ones to be evaluated in these animal models.

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

In-vitro systems have been and will be used by us as replacements wherever possible to examine selected aspects of the targets that we aim deeply investigate. Preliminary studies will be conducted in a range of in-vitro cell assays, using cells relevant to the disease we are targeting. Any substance which is selected for testing in animals will have been examined in a number of these in-vitro tests to ensure that it has the required selectivity at the target site and has the desired affinity (the degree to which a compound may interact with the target) for the target. Where available, comparisons will be made with data obtained from other drugs in the same class to determine which better satisfies the criteria for development. Additionally, within the team, we have access to over a decade's worth of data regarding all the compounds that have been developed for various conditions. If a target has a large body of evidence in the literature, an extensive review will be performed to gain a thorough understanding of the current scientific field.

Why were they not suitable?

Pain is a highly complex process requiring input from many parts of the nervous system and so, for this reason, in-vitro testing alone is not enough to determine if new compounds will be effective analgesics. Furthermore, the use of animals will help determine if novel compounds produce an analgesic effect,

which is impossible to deduce from in-vitro systems. No currently available in-vitro systems can fully replicate human physiology, as stated above. We have fully acknowledged their strengths, reviewed their use, are aware of and appreciate their limitations as these studies may be valuable, but cannot completely replace what behavioural studies can tell us. The behavioural tests that we use have been carefully refined to fit each model and will be chosen based on the scientific outcome expected. Most of these tests mirror the tests used clinically to assess pain in humans. The amount of information we can obtain from carefully designed in-vivo studies is greater and more valuable in translating to clinical studies in its impact than in vitro-studies, as they cannot represent entire physiological systems. In addition, we will use human clinical data where available and use modelling to project occurrences using available datasets.

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 number of animals that will be used throughout the project has been based on numbers used in previous licences of similar nature. We have a statistician available within the organisation who will help us calculate sample sizes using historic data. All experimental designs will be reviewed often to ensure appropriate treatment and control groups are used to maximise scientific output and keep the sample size to a minimum. Where necessary, pilot studies will be undertaken using the least number of animals required. As we expect most of the data generated to be quantitative in nature, relevant statistical tests will be used to analyse all datasets.

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

Our group has adopted a Good Statistical Practice policy, under which all study designs are reviewed frequently by a qualified statistician. These designs are regularly reviewed to ensure best practices. We will also utilise online tools such as the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines, ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines form: a guideline basis for the determination of experimental design (https://arriveguidelines.org/) and the NC3R's (The National Centre for the Replacement, Refinement and Reduction of Animals in Research) experimental design assistant (https://www.nc3rs.org.uk/our-portfolio/experimental-design-assistant-eda). This will ensure we maximise the scientific outcome of our work while using the least number of animals are used and that the correct analysis is carried out to give the best possible interpretation of the results.

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

Where newer study designs and outcomes are being considered, pilot studies will be run to obtain preliminary data, based on which the design of future studies can be determined. Drug treatments are randomised based on pre-dose readings by dividing animals into relevant groups with approximately equal scores. Measurement of treatment effects is carried out with the operator blind to treatments to minimise bias. In all cases, studies will be carried out to the principles outlined in the ARRIVE guidelines.

In the previous licence, we have trialled the use of tail vein microsampling to extract blood for biomarker analysis, which has been successful and subsequently resulted in a significant reduction in the number of animals required for such studies.

Following discussions with the local Animal Welfare and Ethical Review Body (AWERB) and with other organisations, we have realised that there is a drive to reduce the use of surgically prepared sham animals. For this reason, and to reduce animal usage further, we have decided to no longer perform sham surgery in our nerve ligation model. Control groups must be used in compound studies due to the nature of vehicles being variable between each drug developed. Where possible, the control groups can be avoided if a previous study has shown no effects. In addition, we will utilise tissues and samples from our studies to benefit other pain-related projects so we can maximise our scientific output.

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 models we have chosen to use in this licence include those which we believe are minimally invasive to the animal yet will provide us with the most information. We have consulted with clinicians within the organisation to pick the most appropriate models where possible and intend to use those which model important clinical features of the disease states. These models have been extensively studied in the literature and have been validated using compounds which are in clinical use for the treatment of inflammatory and neuropathic pain.

Only one sex of animals (usually female) have been used in most of our previous studies enabling us to control, as far as possible, between experiment variability. In addition, female mice can be group housed throughout the length of the studies whilst males would require rehousing for welfare reasons due to fighting etc., which is not beneficial for our studies. This fighting can induce stress which in itself can affect the pain response. This will also avoid excess animal wastage in the breeding of such animals. Additionally, having both sexes in studies involving behavioural readouts adds an additional complexity in conducting these studies due to interference with the same operator having to handle them and test on the same instrument. In our previous experience of using both sexes, we have had misleading results with behavioural testing. Benefits from the studies are accrued to the wider

population despite only conducting tests in one sex of animals. Discussions with clinical scientists within our group reveal that testing in single-sex groups is not seen as a problem at the proof of concept and efficacy evaluation stage, as covered by the work in this licence. Once a compound enters clinical testing both male and female patients are used and are balanced across treatment and placebo groups. To date, no compound developed from work carried out under the forerunners to this licence has shown sex differences in its effect in clinical trials. However, we will continue to conduct pilot studies to trial different methods of including both sexes in behavioural studies for the entire period of this licence.

Throughout the study period, all animals will undergo daily health checks and be weighed weekly to ensure their welfare. Additional health checks and weighing will be carried out where necessary, for example, after procedures. Due to the chronic nature of the conditions in the clinic, it is necessary to follow the animals for a few weeks to replicate the human disease processes. However, care will be taken to ensure the severity limits set out for each of the models are not exceeded.

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

Since most of the pain indications that our group focuses on are commonly seen clinically in adults, all our studies would prefer to use adult animals. A majority of our studies require identifying pain-like behaviour which can be measured using well-validated endpoints that cannot be assessed in less sentient or terminally anaesthetised animals due to their reduced capacity to perceive pain.

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

All the models in this licence are designed to mimic some, if not most, of the symptoms observed in human conditions and hence some level of pain is inevitable. However, experience gained from previous licences has shown that the level of pain (indicated by the assessed pain-like behaviours) experienced by the animals is not such as to cause any major changes in the welfare of the animals. For example, food and water intake are normal, animals show normal growth curves, and the general observed health and behaviour of the animals are unaffected by the treatments. Often these animals can also be seen climbing grids and running around in their home cage, as early as a few minutes after surgery or dosing. Observations have revealed that the level of activity and general movement seen in treated animals is also no different from that observed in untreated animals. The insults used in the models have been assessed to be the minimum which can be used to cause a significant reproducible biological effect without causing excess adverse events. Measures to reduce animal stress will be undertaken throughout the study period. All animals will be allowed to acclimatise to the study environment and operator before performing any procedures, especially behavioural tests. They will be provided with sufficient enrichment in the cages, which from our experience also helps with recover post-surgery and reducing the incidence of wound licking which may lead to open wounds and infections. In most cases, animals will be socially housed in groups. All animals will be checked daily and weighed weekly, irrespective of the nature of the insult. Based on the model being run, additional monitoring, weighing and health checks will be considered to ensure adequate recovery from any dosing, procedure, or surgery and adherence to humane endpoints. When performing studies involving surgery, we will provide the animals with recovery gel and soft bedding as part of post-operative care. In all new studies undertaken, the level of insult will be assessed before conducting further studies.

Since most studies will involve behavioural endpoints, the animals will be habituated to the instrument and the experimenter before the start of the study.

Previously we have not employed peri- or post-operative analgesia as we believe that the use of analgesics during the development of neuropathic pain following surgery interferes with the mechanisms required to induce the pain symptoms we are investigating. However, we are aware of a small but increasing literature regarding the use of postoperative analgesia in neuropathic models suggesting that no subsequent effects are seen. These studies concentrate on the development of neuropathic hypersensitivity and little or no mention is made of subsequent effects following pharmacological intervention such as the studies covered under this licence. We have therefore conducted pilot studies to investigate the usage of peri-operative analgesia in the surgical neuropathic pain model and observed no difference in the pain phenotype developed of the efficacy of gold standard analgesics used. We will hence employ peri-operative analgesia in the surgical nerve ligation model and local anaesthesia in the inflammatory joint pain models to aid smooth recover from surgery and dosing, respectively. The exception to this would be if any interventions such as tissue collection is planned in the first 48 hours after surgery or dosing of the inflammatory compound, as it may interfere with the scientific outcomes as shown in literature. However, we will consider running pilot studies every time a new target is being investigated to determine the effect of local analgesia on tissue collection and analysis to determine any biological effects. If none are observed, we may consider using local analgesia.

Although we do not expect any major drug interactions, if any novel compound we develop results in alterations of the scientific outcomes due to interaction with peri-operative analgesia, the analgesia regime will be revisited to look for alternatives to avoid drug interactions, while also ensuring adequate post-operative recovery for the animals.

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

We will use the resources available such as the guidance and publications from the NC3Rs, PREPARE and LASA to inform us of any refinements that can be utilised in our studies. Where possible, we will also consult resources such as SYstematic Review Center for Laboratory animal Experimentation to improve the rigour and translatability of studies.

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

All efforts will be made to follow the 3Rs throughout the course of this licence by learning from previous experience and any new studies we may run. We have signed up for the NC3Rs newsletter and will regularly check for latest updates on their website. Where possible, we will also attend any conference or symposia conducted by them and use alternatives or refined techniques. Regular consultations on the latest practical guidance from the Laboratory Animal Science Association (LASA), the Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) will provide additional sources of new recommendations.