Home Office

NON-TECHNICAL SUMMARY

Pharmacological evaluation of imaging agents and development of imaging applications and methods

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Imaging agents, Pharmacokinetics, Biodistribution, Metabolism, Imaging methods

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 aim of this project is

- to evaluate the pharmacological properties of imaging agents,
- to develop imaging methods and
- to investigate applications of the imaging agents.

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?

In the clinic, imaging studies (Computerised Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) etc.) provide the means to visualise and quantify the status of specific molecular targets in living subjects in a minimally invasive manner. Such imaging studies could be for the purpose of research into understanding a disease, diagnosing a disease, assessing the stage of the disease, for treatment selection, evaluating treatment effects, patient selection and grouping for clinical trials etc. As new molecular targets involved in diseases are discovered, the imaging agents that are required for imaging them need to be developed and evaluated in a manner similar to the drug development process, typically involving steps such as

1. Drug design

Lead compound identification

Chemical modification of the lead compound and generation of a compound library

Screening the compound library to select candidate compounds for further evaluation

Production of the candidate imaging agent

2. Biological evaluation

1. In vitro (not in a living organism) evaluation of the candidate imaging agent
2. **Preclinical in vivo (in a living organism) evaluation of pharmacological properties (eg. does it enter the brain?, does it break apart?) of the candidate imaging agent, development of imaging methods and investigation of applications of the imaging agent**
3. Preclinical in vivo evaluation in disease models
4. Toxicological evaluation

3. Translation to first-in-human studies

In this process, this PPL (project licence) aims to address the step 2.2. The overall aim of this PPL is to investigate the pharmacological properties of imaging agents that are often independent of disease conditions and to develop associated imaging methods to be able to use the imaging agents in practice.

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

This PPL will be the first step in in vivo evaluation of imaging agent candidates.

1. Agents with properties that make them suitable for use as imaging agents will be identified from potential candidates. Agents that are not suitable will not proceed to evaluation in disease models with higher welfare concerns.

2. Imaging methods that can be used for evaluation of imaging agents or disease processes will be developed.

3. Questions raised about properties of imaging agents (e.g. Does the imaging agent bind to any other targets?) or imaging methodology (e.g. Can the imaging agent be repurposed for imaging other biological functions?) will be answered.

4. Publication in peer reviewed scientific journals, dissemination of findings, including unsuccessful approaches or non-significant data via open access and through platforms such as F1000 Research

Once an imaging agent is developed with the appropriate characteristics (2-4 years of development), it will be moved on to disease specific PPLs with relevant disease models for further evaluation.

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

In the short term, better understanding will be developed regarding the relationship between the chemical structures of the imaging agents and their biological activity. Novel imaging methods will be developed in order to effectively use imaging agents to study changes that happen in a given disease.

In the medium term, imaging agents developed and used in preclinical disease models will aid the understanding of disease processes and play a part in the evaluation of potential drug treatments that

modify the imaging targets.

These will be circulated in the public domain in the scientific community, guiding future direction in imaging agent development. Thus, in the short to medium term, the beneficiaries are researchers in the imaging and drug development community.

In the long term, studies in this PPL will contribute to development of minimally invasive imaging agents and methods for diagnosis of diseases, choosing the right treatment for the right patient or for development of drug treatments.

For example, imaging agents that bind to specific toxic misfolded proteins (such as tau, alpha synuclein, Huntingtin etc.) deposited in the brain in various forms of dementia will help to diagnose which type of dementia a patient has. Such toxic proteins are formed years before the clinical symptoms develop. When new drugs for removing these proteins are being developed it will help to monitor whether the treatment decreases the levels of the toxic proteins during clinical trials and in practice.

Choosing the right treatment for the right patient is the aim of the project where imaging agents are being developed for brain stem cells called oligodendrocyte precursor cells (OPCs). These cells can develop into the cells that form the myelin sheath which protects neurons. In multiple sclerosis the myelin sheath is damaged and drugs are being developed to convert the stem cells into myelin forming cells. However, these drugs will only be useful for those patients that already have the stem cells. The imaging agent will help to identify which patients are likely to benefit from the drug treatment.

Inflammation is involved in several diseases. However, whether it is the cause or result of the disease is often not clear. Imaging agents that identify markers of various stages of inflammation, especially in combination with imaging for other aspects of the disease will help to understand the disease process better and hence to develop better treatments in the future.

Thus, patients are the eventual beneficiaries.

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

Publication in peer reviewed scientific journals or local, national or international conferences, dissemination of findings, including unsuccessful approaches or non-significant data via open access and through platforms such as F1000 Research.

Collaborations with laboratories and institutions with expertise in different scientific areas to include chemistry, radiochemistry, chemical engineering, pharmacy, physics, clinical imaging and clinical and veterinary medicine have been established to carry out the proposed work.

Species and numbers of animals expected to be used

- Mice: 220
- Rats: 320

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.

Adult wildtype (normal, healthy, without a disease causing genetic change) mice and rats will be utilised.

While some of the preliminary work can be done using in vitro systems, testing the pharmacological properties in healthy, non-diseased rodents is the mainstay of preclinical development of imaging agents and methods. They allow us to demonstrate that the imaging agents and methods have potential for further application in humans as they are a good model for the physiological processes in humans.

They are therefore suitable for assessment of the pharmacological properties of the imaging agents and allows for fast failure of imperfect agents before further application in animal models of disease. For example, PET imaging will tell us in three healthy animal scans if an imaging agent for brain does not pass the blood-brain barrier, thus, it will not be progressed into a disease model. The biological insight gained from such studies in healthy wildtype animals will instead be used to modify the chemical structure of the imaging agent for developing the next series of imaging agent candidates.

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

The procedures carried out on these animals are standardised, generally accepted and will be conducted abiding to humane treatment conditions of animals involved.

Most studies (Protocol 1) will be non-recovery studies where all procedures are performed in anaesthetised (unconcious) animals. They are administered candidate imaging agents (such as small amounts of radioactively labelled small molecules, peptides or proteins to larger structures such as synthetic nanoparticles, radiolabelled cells or other contrast agents) typically via intravenous injection but various routes such as intraperitoneal, subcutaneous or intramuscular may be used instead. Fur may be shaved for surgical placing of cannulas in blood vessels. Agents that interfere with the imaging agent (such as drugs, unlabelled candidate imaging agents, other competing agents) may be administered similarly by various routes to the animals depending on the properties of the individual agent. The animals will either undergo imaging procedures or tissues/blood samples are collected for analysis. Where compounds of unknown properties are administered for the first time, it will be administered in non-recovery studies.

The imaging procedures are carried out under anaesthesia and can last from a few minutes (a 6 minute CT) to a few hours (typical PET-CT or MRI scan of 1-2 hours or rarely up to 4 hours when combining multiple imaging modalities or imaging with radiotracers with longer half-lives). Some studies will involve imaging (eg., CT, MRI) without administration of imaging agents (Protocol 3). At the end of these studies the animals are killed humanely without waking up from their anaesthesia.

Some studies will involve preparing the awake animals for the scan (Protocol 2 and 4). This could be food withdrawal or different feed with or without substances that may interfere with the imaging or pre-treatment via routes such as drink or food, oral administration, or injection routes such as intravenous, intraperitoneal, subcutaneous or intramuscular with imaging agents or agents that interfere with the imaging agent or process depending on the properties of the individual agent. This will be followed by imaging as above or tissue/blood sampling under anaesthesia or urine / faeces collected for analysis while housed in isolation. Where animals are housed in isolation, it will usually be for 1-2 hours or if longer, they will be culled at the end of the isolation period.

A small number of studies (Protocol 2 and 4) will involve multiple scans, on up to 5 occasions, over several days and may involve preparation of animals for scans or sample collections as above (food withdrawal, different feed, administration of interfering substances or imaging agents). Where animals are recovered from anaesthesia, warming boxes will be used to aid in recovery.

At the end of the experiments animals will be killed by a humane method such as overdose of anaesthetic and organs and tissues may be collected for further experiments. Tissues may be frozen and stored for use in future studies.

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

Most procedures will be carried out in unconscious, anaesthetised animals and with careful monitoring there should be no side effects to the animals except for transient discomfort from being anaesthetised.

Preparing the awake animals for studies may cause mild discomfort from being handled for administration of substances via injections (transient discomfort), hunger from short term withdrawal of feed (less than 12 hours, typically 4 hours) or anxiety from rare occurrences of individual housing (1-2 hours if returning to home cage or very rarely up to 24 hours on a single occasion at the end which they will be humanely culled). Some animals will be anaesthetised for imaging on up to 5 occasions which might result in anxiety leading upto the next anaestheisa and during the recovery from anaesthesia.

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

Non-recovery: 64%

Mild: 36%

Moderate: 0%

Severe: 0%

Rats

Non-recovery: 75%

Mild: 25%

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?

It is necessary to conduct studies on animals as the complex interactions between organ systems that may affect the behaviour of the imaging agents in the body cannot be replicated by in vitro studies or computer models.

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

We currently use several tests, depending on the kind of imaging agent, before proceeding to animal studies as follows.

1. Computer modelling will be used where possible to narrow down the library of candidate compounds to those with the best properties.

2. The ability of the imaging agent to penetrate biological membranes which consists of oil-like and water-like parts will be tentatively evaluated by the octanol-buffer partition method. In this method the imaging agent is added to a mixture of an oil-like (octanol) and water-like (buffer) substances and the proportion of the imaging agent distributing into each part evaluated.

3. In vitro binding studies in archived human or animal tissues or cells etc. will be performed where possible to rank the candidate imaging agents. Only agents with good binding properties (often in nanomolar range) will proceed further.

4. The Imaging agent formulation will be tested for stability for example, on the benchtop for typical storage durations, in animal or human plasma or in commercially obtained liver extracts for typical scan durations.

5. Imaging phantoms (objects that stand in for animals) or cadavers will be utilized where possible in order to minimise the number of animals required for developing imaging methods.

Why were they not suitable?

In vitro and computational methods are not fully representative of the biological characteristics of living organisms. These non-animal alternative methods can only provide preliminary and partial information on the pharmacological properties and target selectivity of the imaging agents to allow preliminary screening and selection of the best agents for further evaluation in animals. Only animal models can reproduce the complex interactions between organ systems that may affect the behaviour of the imaging agents.

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 estimated number of animals for each experiment are based on statistical consultation, references from published scientific papers and our previous research experience and published track record in conducting studies on small animal imaging.

Initial studies for the evaluation of novel candidate imaging agents are exploratory and aims to answer questions such as “Which organs do the imaging agent distribute to and in what time frame?”, “Does the imaging agent break apart into radiometabolites after administration into an animal?”. Such qualitative studies typically require about 6 animals per imaging agent and will serve as pilot studies for further evaluation of suitable agents.

When there is a need to statistically compare groups, information obtained from the pilot studies will be utilised to estimate the required number of animals. About 10 animals per group is typical in such imaging studies.

The total number of animals required over the 5 year period was estimated based on the typical number of researchers developing imaging agents within the group and the number of imaging agents that are likely to be evaluated within this time period. Depending on the success of the projects, this number may vary, necessitating an amendment.

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

Experiments will be carefully planned and controlled to reduce variability. All experiments will be designed in consultation with radiochemists, biologists, imaging scientists and animal facility staff via study plans. Imaging data analysis plan will be developed under the guidance of trained imaging

scientists and statisticians consulted where required. Endpoints for which the experiments are powered are discussed and defined before each experimental plan.

In most experiments, the animal is imaged over 1-2 hours, providing information from multiple time points within that time which will reduce the overall number of animals necessary for the research. For example, a group of animals are imaged dynamically from 0 min to 2 hours in order to obtain the time dependent distribution of imaging agents in to various tissues rather than killing different groups of animals at various time points within those 2 hours.

Where possible, blood samples for determination of plasma levels of the imaging agent and radiometabolite levels will be obtained from the same animals that are being imaged. Obtaining all this information from individual animals will contribute to reducing the variability in the data and ultimately leading to a reduction in the number of animals required. To facilitate this, rats are chosen over mice for studies requiring blood sampling. In addition, tissues may be harvested from the animals after the last imaging time point for archival and further in vitro evaluation as required.

For studies where multiple groups are compared, animals will be randomised to the control and treatment groups. Image analysis is performed using automated software which provides opportunity for blinding.

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

Computer modelling and in vitro studies on human or rodent tissues will be used to decide whether the imaging agents can proceed on to animal testing.

Where possible tissues obtained after the animals are killed will be utilised to generate pilot data for future experiments. Being a part of a large biomedical research organisation, such tissue may be obtained from other groups within the organisation via internal mailing lists.

Pilot exploratory studies will first be conducted on small groups of terminally anaesthetised animals to quickly identify unsuitable candidate imaging agents that are then removed from further evaluation as well as select the best imaging agent for further evaluation. Such studies are additionally used to determine the number of animals required to obtain robust data in larger studies.

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.

Wildtype rats and mice will be used in this project. In most cases the animals will be terminally anaesthetised, causing the least suffering.

Dedicated imaging equipment (for example PET, CT, MRI etc.) for use in mice and rats will be utilised in this study. These scanners are designed to closely mimic state of the art clinical scanners and have been developed with imaging of rodents in mind as these models are well recognised to be the ideal preclinical model for this type of setting.

Most studies will involve imaging or blood / tissue sampling in terminally anaesthetised animals. Some studies will involve preparing animals for the scans while awake (for example, by administration of substances, food restriction) and then performing the scans in anaesthetised animals. In some studies, the anaesthetised animals will be woken up after the scan and the scanning procedure repeated on other occasions. These methods cause the least suffering to obtain this amount of data.

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

Rodents are widely recognised as the least sentient species to carry out research of this type. Pharmacological properties are typically evaluated in adult rodents as their organ systems are mature and representative of human adults. The scanners used are miniatures of human scanners and specifically designed for rodents. The inherent resolution of the imaging techniques (for example, about 1mm for PET) necessitates the use of large enough animals such as mice or rats to be able to accurately quantify the imaging data. Rodents allow for the administration of imaging agents in quantities that can be detected by the scanners as well as to sample blood in sufficient quantities (in the case of rats) for establishing image quantification methods.

Where possible, studies will be performed in terminally anaesthetised animals.

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

Scanning protocols can be refined (injected doses, scanning duration, blood and tissue sampling and analysis protocols etc.) based on information gained from pilot studies.

The doses and routes of administration of interfering agents/ unlabelled drugs will be chosen based on established procedure, literature or prior pilot studies to obtain scientifically valid information while not producing adverse effects beyond transient discomfort due to the injections.

During the scans, the anaesthetised animals are placed on scanner beds that are warmed and the animals are physiologically monitored, typically rectal temperature and breathing rate.

In case of longitudinal studies, after each occasion of scanning, animals will be kept in an individual warm environment with access to food and water to recover from the anaesthesia. Anaesthesia will not be re-induced until the animals have recovered from the previous instance of anaesthesia. Where longer scanning durations (2-4h) are required, fluid replacement (warm saline/dextrose) may be administered.

Animal suffering will be minimised by careful observation of the animals undergoing procedures. Guidelines for the assessment of clinical signs will be strictly followed, and experiment on the particular animal or cohort will be immediately terminated and the animal will be humanely killed upon observation of clinical signs.

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

Guidance and best practise will be followed, for example

Refining procedures for the administration of substances.
https://journals.sagepub.com/doi/pdf/10.1258/0023677011911345

Research Animal Training: https://researchanimaltraining.com/

PREPARE guidelines and checklists will be consulted. https://norecopa.no/about-norecopa

NC3Rs website https://www.nc3rs.org.uk/.

Focus will be given to the updated ARRIVE guidelines https://doi.org/10.1371/journal.pbio.3000410 updated in 2020 to design experiments that can be properly executed and reported.

ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) https://arriveguidelines.org/

Other resources from these websites will be consulted.

(LASA) Laboratory Animal Science Association

(AALAS) American Association for Laboratory Animals Science

(FELASA) Federation of European Laboratory Animal Science Associations

(ICLAS) International Council for Laboratory Animal Sciences

(InterNICHE) International Network for Humane Education

Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/laban.1217

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

The organisation has extensive documentation regarding 3Rs including links to publications, portals and websites with information pertinent to all commonly used laboratory animals. This information is continuously updated and distributed via mailing lists. Staff involved in animal handling and experiments undergo rigorous and continued training on these issues and have all these resources available.