NON-TECHNICAL SUMMARY

Provision of an outsourced drug development platform for the treatment of bleeding disorders

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

Bleeding disorders, Haemophilia, Thrombosis

<table>
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<th>Animal types</th>
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<td>adult</td>
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<td>Rats</td>
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<td>Rabbits</td>
<td>adult</td>
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Retrospective assessment

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

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 provide a service to drug discovery clients to support the development of novel drugs for bleeding disorders that have an unmet clinical need.

As part of this work we will improve and refine animal models of disease to ensure they are fit for purpose and are the most appropriate to answer the scientific question when testing new drugs.

A retrospective assessment of these aims will be due by 30 January 2029

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

Despite notable progress in the discovery and development of medicines, there is still a significant unmet need in the treatment of bleeding disorders, affecting 1 in 2000 people in the UK. Bleeding disorders can severely affect quality of life; life-long care including lengthy stays in hospital is not uncommon for patients. Bleeding disorders can have short- and long-term consequences, and treatments can become ineffective if the immune system fights against them. Finding treatments for all aspects of patient care therefore is crucial to improve outcomes for people affected.

This project will generate important proof-of-concept data in the development of potential new test agents (drugs) targeting bleeding disorders, ranging from the most common (haemophilia A), to rarer (e.g. Glanzmann thrombasthenia). These studies will generate vital information that cannot be found without the use of animals. All clients that we work with are developing test agents which will hopefully contribute to helping people with various bleeding disorders, where the need is greatest.
This licence forms part of a suite of project licences that cover the ability to test a potential therapeutic at the early stages; most relevant is a licence that allows us to determine the suitability of a compound (e.g. how it's tolerated, what dose is needed) in healthy animals first.

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

This project will provide important information that aids the progression of new drugs against bleeding disorders through the drug discovery and development process. The information gathered will enable us and our clients to identify the most appropriate treatments to take forward to human clinical trials and enable us to quickly determine which drugs should not be progressed any further.

In addition, this work will increase our knowledge of how new drugs work and will help us to identify changes in the body that occur in response to the drug. We can use this further down the line to monitor responses in humans during clinical trials.

Data from studies under this project may also be used to support patent applications and applications by clients for additional funding. Data produced may also support the design of regulatory studies for clients.

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

Bleeding disorders can impact patients in different ways; some of which can cause too much clotting in the blood, others cause too little. For example, haemophilia patients have bleeding episodes, which can cause blood to gather in the joints and cause major pain. Haemophilia can even be life-threatening if blood loss from the circulation is too high, and prompt treatment is crucial. Other bleeding disorders can cause life-threatening blood clots to form that can cut off blood flow to different areas of the body.

Regardless of the kind of disorder, due to their nature they significantly impact on the quality of life for patients, and both protective and on-demand treatments are needed for most conditions. Current medications, however, can often fall short in being effective for some or all patients, create side effects that also impact on patient care, or require lengthy stays in hospital due to how they need to be given. Further work is needed to develop safe and effective medicines for a multitude of bleeding disorders, and tackle some of the challenges with treating them.

This programme of work is expected to enable us to progress new treatments for bleeding disorders through the key milestones of drug development. Drugs shown to be effective in this project can be advanced into the clinic for testing in patients where they could significantly improve a patient's quality of life.

In the short term benefits will be seen by the client; by the collection of data from programmes that will increase the knowledge of their lead candidates for proof-of-concept (is it possible to use this drug in animals), and efficacy (does the drug treat the disease). Additionally we will continue to increase our knowledge of the best ways to closely mimic the human bleeding disorders.

In the medium and long term, later-stage development of the drug (clinical trials) and if proven effective and safe when taken into the clinic, will see the work under this licence contribute to benefits seen directly by the patients.
How will you look to maximise the outputs of this work?

All studies are designed such that the outputs from each animal are maximised. Expert knowledge is gathered not only from within the preclinical (animal) team performing the animal studies, but from other teams within our company, or our clients' companies. This ensures that all relevant work that has been performed in the laboratory is taken into consideration when designing animal studies. The in vitro (in the test tube) and bioanalysis teams at our company are experts at analysing tissue and blood samples collected from animals and they help with details of sample collection and storage to ensure that the samples are collected and stored in the best way possible. They are also experts at working with small samples, particularly small volumes of blood, meaning that they can often analyse lots of different biomarkers (a measurable indicator of a disease state or other physiological state) and test agent levels from each animal. Any tissues resulting from projects that can be utilised by other projects will be made available. Our company has a very comprehensive sample record system which makes it very easy to assess the tissues we have banked and the conditions of how they were collected and stored.

In addition, we will seek expertise from our established networks, to ensure that we make use of any new knowledge or incorporate better methods of performing animal studies. We will also use these networks to provide information and training to others on the models and techniques we use in our research. We will maintain good communication with managers of the animal facilities to ensure that any tissues from animals being killed that are not required for our work can be made available to other researchers if suitable.

Although there are times where we will not be able to share animal model information (for example, where it would put us at a competitive disadvantage), we aim to publish or share our findings wherever possible, such as control data or where notable refinements have been made in a disease model or procedure.

Species and numbers of animals expected to be used

- Mice: 4000
- Rats: 1750
- Rabbits: 310

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.

Mice and rats are the most common type of animal used for generating ‘models’ of bleeding disorders and for the testing of new treatments. These models mimic areas of the disease in order to provide data on which drugs are likely to work well in humans as a treatment. The immune system of both species has been highly studied, meaning that a lot is already known about how their bodies work, and
the techniques used to mimic the human diseases are well developed. Adult rats and mice will be used for the majority of the work outlined in this project licence as we want the biology of the animals to be fully developed to better represent the patients we aim to treat.

In order to study potential treatments for patients with bleeding disorders, we commonly use animals that genetically mimic the disease symptoms that patients have; for example, in order to study haemophilia A, mice that experience spontaneous bleeding events in the same way that a patient would be crucial for this. Sometimes these symptoms of disease can be severe, with a chance of internal bleeding.

Sometimes, an aspect of a human bleeding disorder is shared more closely with rabbits, for example a protein for clotting that might be present in rabbits but not in rats or mice. Also, larger blood volumes may be needed that rabbits can provide that smaller rodents cannot. Therefore, where justified adult rabbits may be used for carefully selected studies.

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

Most animals will be part of studies that aim to test whether new drugs can prevent or treat the clinical signs associated with bleeding disorders, such as bleeding in the joints or the formation of blood clots. In the majority of experiments the disease will be caused by changes to genes in the rats and mice, which cause them to have symptoms that mimic the human diseases. For example, rats and mice that do not express Factor XIII mimic the disease haemophilia A; like patients these animals can have spontaneous bleeding episodes and require special care. Sometimes these animals may die through bleeding events; although most animals can be humanely killed beforehand, sometimes it is unavoidable for some kinds of bleeding events such as significant internal bleeds. Similarly, handling the animals in order to perform the experiments can also trigger bleeding events. Taken together this is why severe severity is needed for some of the animals that mimic the symptoms of human bleeding disorders.

As part of the study, animals will be dosed with drugs over a period of days or weeks. Dosing may take place on a daily basis but this will vary depending upon the drug. Drugs will be most commonly given by the intraperitoneal (i.p., into the body cavity), subcutaneous (s.c., under the skin) and oral (by mouth) routes and less frequently by the intravenous (i.v., into a vein) route. For i.p., s.c. and oral routes, conscious animals will be held securely by a trained researcher and the dose administered. For i.v. dosing, animals will be placed briefly in a specially designed rodent restrainer. The animal may be placed into a purpose-built warming cabinet for up to 10 minutes prior to restraint. For rabbits, specific restraint methods will be used; either the most suitable and refined manual method by researchers, or a specially designed device, whichever is most appropriate for the procedure.

Blood samples may be collected during some studies to measure levels of the drug or to assess how well the drug is working. Blood samples are usually small in volume and are most often taken from a vein in the tail, but sometimes from a vein in the leg (saphenous) or, in rabbits, the ear. Sometimes, tests will look at how the animal moves, such as balance and gait analysis (how the animal walks).

The majority of the data will be collected at the end of studies, where animals will be under deep terminal anaesthesia, in order to perform tests on how effective a test agent is at stopping bleeding, or
whether it helps form a blood clot. Here, animals are asleep, unaware of any pain, and do not regain consciousness. The animals will then be humanely killed while still under anaesthesia.

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

Mice and rats with a genetic bleeding disorder, such as those that have clotting Factor VIII or IX missing, are models of human haemophilia A or B. As in humans, these animals are likely to experience spontaneous bleeding episodes that may be painful, and these animals are not expected to live as long as normal rodents.

In many cases research is carried out in animals that are anaesthetised, and from which they won't recover. This means that the animal does not experience any pain or suffering from the procedures carried out, only from the process of being anaesthetised. Under these conditions, we measure blood loss, and the time to stop bleeding from a cut surface (the tail in rats and mice, or the ear vein in rabbits). This mimics that seen in humans where cuts are made in the skin, and how quickly bleeding stops is timed. To induce blood clots we expose blood vessels in anaesthetised animals and cause damage to a vein or artery by adding a chemical or disturbing it with a focused laser beam. The resultant clot can then be either be looked at under a microscope and measured, or it can be removed and weighed. We can measure the time it takes for a clot to block the blood flow through a vessel. Giving a drug before injury can test its effect on bleeding and clot formation. In some cases drugs are given to the animal whilst it is awake and small blood samples taken to measure levels of drug in the blood, but these procedures are not expected to cause anything other than minimal pain or distress.

In some of the tests, such as when joint bleeds are created, we need to assess how much pain the animal is in, to see if the potential drug is making a difference. This means that some pain may be experienced by the animal (possibly over a few 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)?**

**Mice:**

- Non-recovery - 25%
- Mild - 67.3%
- Moderate - 3.8%
- Severe - 3.9%

**Rats:**

- Non-recovery - 57.1%
- Mild - 40.9%
Moderate - 2%

Rabbits:

Non-recovery - 16.1%

Mild - 79.9%

Moderate - 4%

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

- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 30 January 2029

The PPL holder will be required to disclose:

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

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In order to understand the effects of potential drugs in treating disease, the whole "system" must be studied. Animals enable us to mimic the whole biological system, allowing us to study how the vascular (blood-carrying) system interacts with other cells and organs. It is not possible to fully study this in isolated cells and/or organs.

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

Our company regularly uses a range of in vitro (in the test-tube) methods utilising cells to understand how a novel test agent might affect the cellular functioning of those cells. We can sometimes use cells from patients that might help to understand how effective the drugs might be. From these experiments we can prioritise test agents and only take forward those that have the desired effect and, therefore, look the most promising for the treatment of bleeding disorders. We also use computer modelling "in silico" methods to see how drugs might interact with targets in the body. However, this does not fully answer the research question and studying the whole animal is still necessary, due to how the different body systems work with each other.

Why were they not suitable?
None of the alternatives mentioned can replicate the complete model of the human vascular system, which is required to accurately evaluate the impact of a test agent on a bleeding disorder. In addition, cell-based work does not test the effects the body might have on a test agent, for example how it gets into the blood, travels around the body and how it is removed from the body. No alternative is currently available that can replace the need to test potential therapeutics in a live animal.

A retrospective assessment of replacement will be due by 30 January 2029

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

**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?

We have analysed the number of animals used previously on projects and typically how many animals it takes to fulfil each kind of study, using the most up-to-date experimental methods. This was then combined with a prediction of likely demand of future projects over the lifespan of the licence; this is based on existing relationships with clients, current interest from business development, and the historical likelihood of programme types to make it to the in vivo stage (i.e. pass the stop/go stages from preceding non-animal studies). We have had at least two client programmes concurrently for the length of the preceding licence, so studies are generally ongoing.

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

We have extensive experience in the design of experiments of the types in this project, which has given us confidence in the number of animals required to ensure that no animals are used unnecessarily, but also that the data generated is robust and reliable. We regularly refer to the PREPARE guidelines (https://norecopa.no/PREPARE) and make use of the NC3Rs Experimental Design Assistant (https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction) to ensure that we are using the correct number of animals for every study. We also draw upon any formal training we have had, such as courses from FELASA.

When designing experiments to look at the effect of novel test agents, where the test agent has not previously been dosed before, we look at published literature or client data to determine the variability observed with similar test agents. We can then use the NC3Rs Experimental Design Assistant, or our
own in-house developed tool to help determine the most appropriate group size. In addition we have several contacts to draw upon for advice, for example former colleagues.

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

If a new agent is to be tested or refined, small initial experiments (pilot studies) may be conducted first to make key adjustments before proceeding with the larger experiments. This ensures that the correct number of animals are used when experiments are performed in full.

Data from pilot studies and previous experience are used to ensure that the numbers used are as low as possible, without compromising the reliability of the data. Within our company, a member of the wider team has generated a tool for performing power calculations and can be consulted as necessary to assist with study design. This is regularly compared and checked against similar peer-reviewed versions and adjusted where necessary.

Wherever possible, our in vivo (animal) scientists will be blinded to the treatment status of an animal, therefore reducing bias. This enables more reliable information to be gathered from a smaller number of animals. Those who carry out analysis on samples (e.g. blood or tissues) collected during the study are also blind to the treatment status of the animal where possible.

Baseline data (e.g. bodyweight) are recorded and animals are randomly assigned to treatment groups so there is no difference between the groups at the start of the study.

Good planning ensures that within any series of studies we can control for variability that might be introduced by external factors. To limit this variability we use animals of a similar age/weight range, test batches of test agent in the lab first, use the same source of animals and reagents, keep records of all observations made and standardise as many components of an in vivo study as is practicable.

Where possible, we will coordinate with other groups to share tissue including post-mortem tissues to reduce overall animal numbers.

Where genetically altered animals are required, these will usually be provided by our internal breeding projects, which will ensure that animals are bred efficiently using as few animals as possible by communicating need across projects with colony managers. For this we plan out breeding strategies using a projection similar to the Jackson Laboratory breeding colony worksheet. Where animals are obtained from external sources, only the number of animals required for the study will be purchased or imported.

A retrospective assessment of reduction will be due by 30 January 2029

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

Due to the nature of bleeding disorders and in order to study them, commonly animals that show the same symptoms must be used. For example, rats and mice that have the same (or similar) gene mutations to haemophilia patients are used (missing crucial clotting factors). These animals bleed more easily when injured and can also have spontaneous bleeding events. It is important to use these animals, but there are ways to manage them that reduces overall harm while they are in studies:

- Haem A, Haem B, and Glanzmann mice that are used to study bleeding disorders need special daily checks that monitor for bleeding events; this can pick up any bleeds that can be treated with a powder (stypic) if it is on the skin, or that need further monitoring. They also require a change from the usual bedding to a softer variety and the removal of certain enrichment due to their activity levels and propensity to bleed when knocked.

- Haem A rats require monitoring over and above regular colony checks; rats vulnerable to having bleeds (homozygous) are given a thorough health check, checking all over their bodies for signs of bleeds under the skin. Signs of bleeds within the body (internal bleeds) are also observed, by for example pallor (very pale extremities like the ears). Their body weight is checked and recorded if there are any signs of ill health. The daily checks pick up bleeds early on, meaning that appropriate action can be taken very soon in the progression of a bleed. Rats with a bleed can be given a treatment to help the blood clot (Factor VIII or Factor VIIa), and any suffering can be eased with pain relief given in Nutella (this means the rats will readily eat it from a syringe tip). Generally, the first treatment and any pain relief reduces any suffering to a minimum and the bleed disappears over a day or two. Animals will be housed in social groups where possible and provided with an enriched environment in order to minimise stress. Animals will be monitored regularly for signs of adverse effects including unexpected bleeding episodes, body weight, activity, responsiveness, condition of coat and posture.

When studying bleeding in joints in mice, we briefly pierce the knee with a very small needle while they are asleep. This method used is very effective at triggering bleeding, while not needing intrusive surgery to change the joint.

Sometimes rabbits are used for bleeding disorder studies. This is because they might have similarities with humans that rodents don’t (how some clotting factors work), or that they can provide a lot more data that a mouse or rat can (for example blood collection over several sessions that would cause problems to smaller animals).

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

Adult rodents and rabbits are the lowest species of mammal that allow us to adequately study the complexities of human bleeding disorders. Although terminal anaesthesia is commonly used for bleeding disorder models, some studies can often take weeks, based on how a disease progresses, or
if preventative treatments are being tested. Therefore, although we estimate that a third of our studies will be under terminal anaesthesia, this is not always possible. It is also important that we are able to monitor the behaviour of the animals in a conscious state where relevant. This allows us to monitor for adverse reactions to any new test agents administered and also how the diseases (particularly for joint bleeds) affect behaviour and movement.

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

Animals will be housed in a purpose-built, modern and well-equipped facility that is free of disease-causing organisms such as bacteria, viruses and parasites. They will have access to food, water and where possible items that enhance their environment, such as tunnels, chew sticks and two-storey levels to climb on. Our company staff and the animal care staff are competent in rodent and rabbit welfare and will ensure that animal suffering is minimised. We aim to house animals in groups to promote normal behaviour. However, aggressive behaviour can occasionally result in animals being singly housed to prevent injury.

Each project has a dedicated project manager and a team of highly experienced researchers. This enables us to combine years of knowledge and experience and tailor strategies to refine experimental design as well as the procedures themselves in order to minimise harms to the animals. Open and regular communications with other managers throughout the Establishment alongside unit technicians, Named Animal Care and Welfare Officers (NACWOs) and Named Veterinary Surgeons (NVS) further enables relevant and specific care for our studies and to identify any new and better methods that could be utilised.

To minimise distress in joint bleed mice we will follow measures that include the provision of soft sawdust litter to reduce any irritation on walking, the use of non-tangling nesting material and long nozzles on drinking bottles if movement is impaired. Nutri and hydrogel will also be provided if needed.

All procedures are performed using the smallest needle possible. The lowest volume of blood needed for experiments is determined prior to the study starting to ensure the smallest amount of blood is taken as possible from animals during blood sampling. Injection sites will be monitored for signs of redness, swelling and infection.

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

We use PREPARE guidelines for the planning of animal experiments; these complement the latest version (2020) of the ARRIVE guidelines that are a checklist of important information to include when reporting animal research. Taken together these ensure that animal studies are reproducible, and as translatable to human diseases as possible.

We will also consult Laboratory Animal Science Association (LASA) publications for more general topical advice.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
When designing animal studies we consider the appropriate guidelines, including the guidance from The National Centre for the 3Rs (NC3Rs), Laboratory Animal Science Association (LASA), and the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines. This guidance will influence our study design. Searches for publications will also be performed on resources such as norecopa.no to check that relevant 3Rs information is found.

**A retrospective assessment of refinement will be due by 30 January 2029**

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?