



Home Office

## 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

thrombosis, anticoagulants, haemophilia, platelet disorders

## 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
- Required at inspector's discretion

# Objectives and benefits

**Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.**

## **What's the aim of this project?**

When a blood vessel is damaged twenty specialist proteins in the blood (clotting factors) combine with cells in the blood (platelets), which makes the blood sticky and bleeding eventually stops (haemostasis). This process is complex and tightly regulated, but if this regulation is abnormal then unwanted blood clots (thrombosis) can occur inside arteries and veins, leading to a clinical illness, such as stroke or deep vein thrombosis, or unwanted bleeding can occur, as in patients with haemophilia. The development of drugs to treat thrombosis and haemophilia forms the basis of this project licence.

The most common medicines prescribed for unwanted clotting of the blood are anticoagulants, sometimes referred to as 'blood thinners'. Warfarin is still one of the most widely prescribed anticoagulants in the NHS. However, the effects of warfarin have to be carefully monitored. If levels are too low then the beneficial effects are not seen and if they are too high then the anti clotting properties put the patient at risk of bleeding. Newer anticoagulants have been approved, but their use is still associated with an increased risk of bleeding, and bleeding complications can be life threatening. A bleeding tendency caused by anticoagulants is not a side effect but the main effect of the drug, and the leading question in the development of anticoagulant drugs has been 'Is it possible to make a potent anticoagulant without a bleeding risk?' Testing this hypothesis with new drugs that are being developed is one of the aims of this new project licence.

About 6,000 people in the UK have haemophilia. They don't have as many clotting factors, so their blood takes longer to clot. As a consequence they may have nosebleeds that take a long time to stop; wounds that bleed for a long time; skin that bruises easily; or pain or stiffness in the joints from internal bleeding, which can cause loss of mobility. Haemophiliacs are injected with medicines that replace the appropriate missing clotting factor. The biggest disadvantage with these medicines is that some people develop antibodies in their immune system, called inhibitors, which make the medicine less effective. These patients then need to take further medicine to overcome this, but these drugs are not very effective. Inhibiting the production of our bodies' own natural anticoagulants that normally act as brakes to limit clotting is an alternative approach we are investigating. Gene replacement therapy is another. This is an experimental technique where genetic material (DNA) is introduced in to a patients cell to compensate for an abnormal gene or to make a beneficial protein, such as missing clotting factors.

A very rare condition called Glanzmann's thrombasthenia (GT) is caused by a problem in cells in blood namely platelets, these cells rush to a point of injury (such as a cut) to stop bleeding. As with haemophilia, the body can eventually develop inhibitors that stop effective treatment. Fortunately, it's been discovered that a treatment used for Haemophilia also works for GT, which greatly improves patients' hope of treatment. However, any current treatment still relies on long stays in hospital to get the drugs or replacement platelets given slowly over time into a vein. The aim of this part of the project is to investigate a new treatment that can be injected under the skin by the patient themselves, which would significantly increase patient quality of life.

## **A retrospective assessment of these aims will be due by 30 March 2024**

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.**

### **What are the potential benefits that will derive from this project?**

The short-term benefit will be to further our understanding of the blood coagulation process and whether our approaches to developing new medicines are likely to work when tested in humans. In the long term, successful drugs will offer distinct advantages over current medicines. Blood clots that form in arteries are the most common cause of strokes, and strokes can lead to severe disability or death. It is expected that by 2050, 16 million people will be affected in this way and will cost the NHS £2.2 billion per year. Severe disability or death, due to blood clots that form in one or more deep veins, also affects approximately 1 in 1000 adults each year, especially as they age. Deep vein thrombosis is currently considered the commonest avoidable cause of hospital death. The diagnosis and management of abnormal blood clotting is therefore very important. About 6,000 people in the UK have haemophilia. There is no cure for haemophilia. The current injectable artificial factor medicines have their limitations, including high cost and restricted availability. But the biggest disadvantage is the development of inhibitors, which poses special challenges. The healthcare costs associated with inhibitors can be staggering because of the amount and type of treatment product required to stop bleeding. Haemophiliacs who develop an inhibitor are twice as likely to be hospitalised for a bleeding complication, and are at increased risk of death. Developing a drug that doesn't lead to the development of inhibitors, will offer advantages over current medicines. A drug that is cheap to produce will especially benefit the majority of haemophiliacs who currently have no access to effective therapy and have a life expectancy of only 10 years. Research into gene therapies for haemophilia has the potential to offer a 'one off' treatment by enabling the patient to generate their own missing clotting factors, rather than being given multiple injections of these factors. New medicines that go on to be tested in clinical trials, will guide the improvement of animal models (that are comparable to the human disease) these medicines have been tested in, and justify their value as experimental tools.

### **Species and numbers of animals expected to be used**

#### **What types and approximate numbers of animals will you use over the course of this project?**

All our animal studies use the lowest species possible to answer the scientific question being asked (predominantly rats (5700, which includes genetically altered animals that are models of haemophilia) and mice (12100, which includes genetically altered animals that are models of haemophilia and Glanzmann's thrombasthenia), but for some targets we may need to use rabbits (210).

## Predicted harms

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

**In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?**

In many cases research is carried out in animals that are anaesthetised, and from which they won't recover. Under these conditions, we measure blood loss, and the time to stop bleeding from a cut surface, of 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, looked at under a microscope and measured, or it can be removed and weighed. 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.

Mice and rats with a genetic bleeding disorder, such as those that have clotting Factor VIII missing, are a model of human haemophilia A. 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. The liver makes clotting factors, such as FVIII or FIX, so when measuring how well a novel therapy, such as gene replacement therapy, is likely to work, we may need to compare different routes of administration. This could include giving it directly into the vein that runs into the liver (called the hepatic portal vein), through a surgical procedure carried out under anaesthesia with recovery. If this is successful, then an alternative method to deliver the vector to the liver, we may explore, is by injection into the spleen. The spleen is an organ that lies close to the liver and its vein drains blood into the hepatic portal vein. Therefore any vector that is injected into the spleen will end up in the liver, but to prevent the gene becoming incorporated into the spleen on its way through, it may be necessary to remove the spleen. Another method of gene replacement therapy that is emerging as being of clinical importance is to inject the genetic material in a large volume at high speed, called hydrodynamic delivery (HD), which maximises delivery of the gene to the tissues. In mice HD of genetic material, injected via the tail vein to reach the liver, causes the heart to become over filled and the liver to expand but both return to normal. Animals may lie flat immediately following the injection but they quickly become upright again and move about. After this time they may become subdued but responsive to stimuli for about an hour before their usual behaviour returns. To minimise any pain animals are likely to experience we will treat them with pain killers or to kill them by a humane method, if they show signs of distress that do not get better after a short period of time. At the end of any study all animals are humanely euthanised.

**A retrospective assessment of these predicted harms will be due by 30 March 2024**

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 why you need to use animals and why you cannot use non-animal alternatives.**

Blood coagulation is an incredibly complex process that is under tight regulatory control. Blood flow plays a significant part in the coagulation process, therefore to understand haemostasis fully, and it normally functions in relation to human bleeding disorders, it is important to use the whole animal.

**A retrospective assessment of replacement will be due by 30 March 2024**

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 you will assure the use of minimum numbers of animals.**

As part of our research, we use a statistical approach to calculate the minimum number of animals needed for our experiments. We also use appropriate statistical tests to compare the results we get between different treatment groups. Specialist scientists, who are experts in the field of statistics are available to help us with our approaches. In this way we can be confident that any effect of a drug we see is a real effect and did not happen by chance. This means we may not have to repeat experiments.

Pilot studies that are used to refine procedures and to discover potential problems before the main study begins, uses relatively small numbers of animals based on experience and judgement, but numbers are large enough to provide needed estimates for future sample size analysis. Good planning ensures that within any series of studies we can control any variability that might be introduced. This includes using animals of a similar age/weight range and that have had a similar environment throughout their lives; assigning animals to treatment groups using a tool that selects them at random; testing different batches of test agents in non-animal experiments first; using the same source of reagents; keeping records of observations made and standardising as many components of an animal model as is practicable. Wherever possible, scientists involved in every aspect of an experiment are unaware of which animal received which treatment, and are only made aware of this when all the data has been gathered. This reduces any potential bias that could influence making the effect of a new drug look better than it really is. If an appropriate genetically altered animal is not available commercially, then we manage our breeding programmes to minimise animal wastage. In most cases most excess animals are used to provide blood or tissues to support our work, or are offered to others.

Exploring emerging technologies such as HD, to maximise the delivery of molecules (that are not permeable to the cell membrane) to the liver or other tissues, has the potential to reduce the number of animals needed to achieve a positive result when compared to other methods of delivery.

Where GA animals are used, the breeding strategies are optimised to generate the required number of GA animals with the lowest possible number of wildtype and other animals of undesired genotype. With the Haem A rats, these had previously been bred using a heterozygous x heterozygous breeding strategy. We have trialled using homozygous males for breeding, which has been successful and has increased the proportion of homozygous animals generated and reduced the number of excess animals. Wherever possible, excess animals generated as a result of the breeding process are used for other studies, rather than purchasing wildtype animals from external breeders.

### **A retrospective assessment of reduction will be due by 30 March 2024**

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**

**Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.**

The ideas behind the novel drugs have come from observations made by doctors in patients with bleeding disorders. The animal models of bleeding disorders outlined in this application have been carefully selected to mimic certain aspects of the blood coagulation process (seen in human diseases) that our new drugs are likely to affect. These models were refined under our previous licence to generate robust working procedural methods, but we will continue to refine these models new ideas are generated. We will mostly use mice or rats but, for some studies we may need to use rabbits. This is because some of the factors which control clotting are not exactly the same in rats and mice as in humans, but they may be in rabbits. Genetically altered rodents that have a bleeding profile, such as haemophilia A mice and rats, or Glanzmann's thrombasthenia mice, are expected to show an increased risk of bleeding. These animals do require careful handling to minimise the incidence of bleeding, especially when carrying out a procedure, such as an injection, and scruffing for injections or blood sampling will be kept to a minimum. We plan to carry out pilot studies to investigate the use of light gaseous anaesthesia when carrying out injections. An alternative, refined method to deliver genetic material to the liver could be by injection into the spleen, if the genetic material is well-suited to be given in this way. The surgical procedure needed to access the spleen is simpler than that to access the hepatic portal vein, so animals are likely to recover more quickly. If giving the genetic material by HD is more successful than giving it via the spleen or portal vein then this will negate the need to carry out a surgical procedure, and animals may not need to be kept for as long (we expect to the effects of HD to be seen within 4-5 hours, rather than days when given into the spleen or portal vein). We always use best practice for husbandry and special considerations are made where needed, such as providing our haemophilia and Glanzmann mice with extra surgical bedding to help minimise the incidence of spontaneous bleeding. We are now experienced at detecting and treating spontaneous bleeds in haem A rats. We are able to spot these bleeds early during daily checks, to ensure that bleeds do not progress in severity and that the rats do not experience unnecessary pain. We have a good understanding of when a bleed requires multiple treatments and where swelling remains and takes a

few days to recede but the bleed has already resolved, to avoid unnecessary restraint and injection. Where pain relief is required, this is administered via formulation with Nutella, to further reduce the handling required and to avoid further injection. The rats are exposed to Nutella in the cage at weaning, so that it is a familiar substance if pain relief is later required. Animals are housed in social groups as far as is practical, and if bred in house, then this is done to exacting standards. March 2021: We have now developed a method of using small earpieces to find out whether the rat have the gene we're looking for, which reduces the risk of causing any bleeding. We seek out new guidelines and information from sources such as the Home Office, the RSPCA, the laboratory animals science association (LASA) and the national centre for the 3Rs (NC3Rs) to maintain awareness of advances in animal welfare.

**A retrospective assessment of refinement will be due by 30 March 2024**

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?