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NON-TECHNICAL SUMMARY

Platelets as a therapeutic tool

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Platelets, Clotting, Bleeding, Transfusion

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is 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?

To fully test and understand how good platelets (blood cells that help stop bleeding) made from stem cells are at stopping bleeding. This will inform future studies and provide pre-clinical data for clinical trials.

A retrospective assessment of these aims will be due by 17 April 2028

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?

Platelets are blood cells whose primary function is clotting. There is a huge unmet need for the transfusion of platelets because at the moment we rely solely on blood donors. We are generating platelets from stem cells in the laboratory and need to test how good these platelets are for transfusion and clotting in mouse models. We need mouse models to fully understand how they work in a live mammalian system because testing in a laboratory is not complex enough to completely reproduce what happens at the site of bleeding in a human.

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

We are generating platelets (involved in blood clotting) from stem cells, some which contain additional factors that are involved in blood clotting and others which contain drugs which can be used in patients. In particular, platelets that will not be rejected by the recipient’s immune system. We expect to produce several high impact publications from this project from the basic biological science we need to develop to push this forward.

In addition, these projects will provide experimental data that will be necessary for approval for the first-in-human studies. We also expect that the data will form the basis of further funding that will be necessary for the translation to human studies.

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

The immediate benefit of this project will be to patients with excessive bleeding because their platelet count is low, or their platelet do not function well and are therefore in need of platelet transfusion. There is no doubt that making blood cells in the laboratory, particularly platelets, will have a huge

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impact on transfusion medicine. At the moment donor-derived platelets are the only option for transfusion (280,000 units of platelets are used in the UK every year) but this has issues: supply, exposure to donor-derived infections and rejection by the patient of donor-derived platelets.

This project will deliver knowledge and preclinical data that would push forward the opportunity to deliver platelets made from stem cells into patients. The ability to produce platelets that are not rejected by the patient and/or contain specific therapeutics (such as clotting factors) provides us with the opportunity to develop 'personalised' cell therapies. Platelets are particularly attractive as they do not contain a nucleus (they are unable to make their own DNA) and are therefore less of a risk in terms of their ability to form cancer after their administration, when compared to other cell therapies derived from stem cells. This project will provide evidence that platelets can be used as ‘drug delivery vehicles’ targeted to the site of interest. The technology developed in this project will be also relevant to other researchers aiming to produce either blood cells in the laboratory (such as red blood cells) or other organ cells in the laboratory (liver, pancreas, heart muscle).

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

We have a number of successful collaborations with academic groups in the UK, the UK transfusion service (NHS Blood and Transplant) and academic groups in Europe. We are in active collaboration with groups that can produce stem cell derived therapies at clinical grade for human trials. We will disseminate the output from this work at various conferences, both in the UK (The Platelets Society Conference) and internationally (ISTH, ISBT, EHA, ASH) and in peer-reviewed publications. This group is very active with Public Engagement.

Species and numbers of animals expected to be used

- Mice: 4680

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 are the lowest order of mammals that we can use to study the platelets in a whole mammalian system. In general we are using adult mice, so that their bodily systems are fully mature.

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

Superovulation (ovulating more eggs than usual) - Female mice will be given agents to increase egg production twice. Embryos and blastocysts will be removed under terminal general anaesthesia.

Embryo recipients - Embryos and blastocysts will be implanted surgically or non-surgically into the reproductive tract of a mouse made pseudo-pregnant by mating with a sterile male. All surgically

implanted mice will be killed by a Schedule 1 method. Non-surgically implanted mice will be killed by a Schedule 1 method or kept alive for potential re-use on a breeding protocol.

Vasectomy (cutting and tying of the tubes that carry sperm) - Male mice will undergo a vasectomy under anaesthesia. These males will then be used to induce pseudo-pregnancy in embryo recipients.

Analysis of blood cell function - Mice will be bled from a superficial vessel, administered a cell modulation agent (a maximum of three times per day for a maximum of 15 days) and then bled from a superficial vessel on a number of occasions (several times in one day, or over the period of 28 days). They will then be exsanguinated (terminally bled) under terminal anaesthesia and killed by a schedule 1 method.

Tail vein bleeding model -mice will be injected with a cell modulation agent (an agent that alters cell function) (a maximum of three times per day for a maximum of 15 days). Mice will then be injected once via the tail vein with platelets made in the laboratory. Terminally anaesthetised mice will have the tip of their tail (<5mm) cut off and the amount of blood loss will be measured.

Myeloablation (irradiation) - mice will be irradiated with a low as possible degree of radiation in a split dose. They will then be given blood cells once via a tail vein injection. Mice will then be bled from a superficial vessel on a number of occasions (several times in one day, or over the period of 28 days), before being bled from a large vessel under terminal anaesthesia and killed.

Myeloablation and the tail vein bleeding model - Mice will be irradiated in a split dose and then administered haematopoietic(blood)/terminally differentiated cells once via an intravenous injection. Mice will then be bled from a superficial vessel on a number of occasions (several times in one day, or over the period of 28 days) before undergoing the tail vein bleeding model. Mice will then be exsanguinated (terminally bled) under terminal anaesthesia and killed by a schedule 1 method.

Transfused mice and analysis of blood cell function using pulsed laser microscopy (the use of a laser to induce injury)- Mice will be administered a cell modulating agent (an agent that alters cell function) (a maximum of three times per day for a maximum of 15 days) and bled from a superficial vein on a number of occasions (several times in one day, or over the period of 28 days). They will then be administered samples containing haematopoietic/differentiated cells once or a control substance before being terminally anaesthetised and undergoing pulsed laser microscopy.

To analyse blood cell production and function post-splenectomy (removal of the spleen) - Mice may be administered a cell modulating agent (a maximum of three times per day for a maximum of 15 days), bled from a superficial vessel (several times in one day, or over the period of 28 days) and splenectomised. Mice will then have follow-up bloods taken from a superficial vessel (several times in one day, or over the period of 28 days). Mice will then be exsanguinated under terminal anaesthesia and killed by a schedule 1 method.

Myocardial infarction (MI) (heart attack) - Mice will be subject to general anaesthesia and will be ventilated. The chest will be opened and the major arteries which supply the heart will be blocked to induce an MI. The wound will then be closed, and pain killers will be given. During recovery mice will be housed in a warm cage for close monitoring. During the initial period of recovery from operation the animals will be checked every 10 min, then every hour until they can move freely when lightly disturbed. The function of the heart may be measured with echocardiography (ultrasound) during this

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recovery period. Mice will receive a transfusion of cells via the tail vein post MI once. At the end of the protocol, mice will be humanely killed with Schedule 1 method.

We have added an extra protocol (Protocol 10, non-recovery) where a bleed from the inferior vena cava under terminal anaesthesia is compulsory, with animals then being killed humanely. The reason for this is that we sometimes need to get large amounts of blood (larger than that which can be obtained by a tail vein bleed) to isolate platelets which may then be injected into other mice or used for our in vitro experiments. All animals are under deep, terminal anaesthesia, they will not undergo any suffering.

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

Animals which have a weakened immune system (immunosuppressed) may display a higher rate of illness. Mice may lose weight for up to four days following irradiation. Injections into the bone marrow (Intraosseous) may rarely result in lameness, which may cause more than transient discomfort. The main adverse effects following removal of the spleen (splenectomy) are pain after surgery, bleeding during surgery, reopening of the surgical site and infection of the surgical site. The main adverse effects of surgery to induce a heart attack (myocardial infarction) are sudden death due to an abnormal heart rhythm (ventricular fibrillation), stoppage of the heart beating (cardiac arrest), heart failure, pain after surgery, bleeding during surgery, reopening of the surgical site, surgical site infection and developing high blood pressure in genetically altered animals. Drugs given to cause a chemical injury to the blood vessels which supply the heart may have undesirable effects. In some circumstances we may not use non-steroid anti-inflammatory drugs to control pain, but other pain relieving drugs, as these may alter platelet function. Mice in which we will cut the tails for the tail vein bleeding model will be anaesthetised and therefore will not feel pain. It is important for us to carry out the tail vein bleeding model so that we can test the cells that we made in the laboratory in a living system, where there are a number of other cells, as we cannot fully mimic this in the laboratory setting. Some mice maybe injected with a drug that alters their cells (cell modulating substance), for example this drug may bind to the mouse’s platelets and reduce them before we give them platelets that were made in the laboratory. This is unlikely to cause harm which is more than minor and transient.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The expected severities in this licence are:

Mild - 76%

Moderate - 16%

Severe - 4.3%

Non-recovery - 4.3%

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

- Killed

A retrospective assessment of these predicted harms will be due by 17 April 2028

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?

This work will be supported by studies in the laboratory of human platelets and megakaryocytes (MK) (the cells which platelets are derived from) wherever possible, including the use MKs derived from stem cells. However, human platelet and MK studies cannot fully replace the animal studies in this project for three reasons.

1) Megakaryocytes represent only 0.01% of all bone marrow cells and therefore we cannot get enough from human bone marrow samples. Although MKs can be grown from human stem cells in the laboratory, studying the way they work (particularly how platelets are released) cannot be done entirely in the laboratory due to our inability to reproduce the complex bone marrow environment that provides vital clues to how MK's mature. The variability in different donors (such as genetic variations) can influence results limiting reproducibility. This is not an issue when using mice colonies where genetic variations are minimized.

2) Although our ability to produce platelets in the laboratory from cultured MKs has markedly improved, it remains to be proven that these platelets are truly similar to fresh platelets in terms of the way in which they work and how effective they are at stopping bleeding (the proposed work plan is looking at addressing this issue). Therefore, in order to study how platelet function (how they work) is affected by the different genetic mutations we have to resort to the study of platelets isolated from fresh blood. Platelets do not have a nucleus (where DNA is made), which means that standard methods of altering genes cannot be used. Human blood can potentially, but regular access to specific patient samples would severely limit the work.

3) Recovery, survival and function (clotting/tissue repair) in the mammalian system of transfused cells is regulated by a very complex environment. These can only be reproduced within the blood environment of a living organism. Mouse studies will provide essential proof-of-principle data that will be necessary in order to gain approval for human studies of blood cells produced in the laboratory.

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

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We are unable to recreate the complex 3-dimensional bone marrow environment in the laboratory. Although we can mimic some of these characteristics, particularly using scaffold structures, these experiments are usually 1-dimensional in what they can provide to the cultured cells vs live bone marrow.

Why were they not suitable?

Chambers which enable blood to flow through can be used to test the formation of blood clots; however, they do not mimic the environment found in blood vessels, particularly the way blood flows in a pulsatile manner (with a push and then ebb) and interaction with cells lining the blood vessels. In addition, there are no models that can mimic the interaction of platelets with other organs in the body.

A retrospective assessment of replacement will be due by 17 April 2028

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?

Pilot studies with a minimum number of animals will be carried out in order to assess the validity of each experiment and to refine group size estimates. If the pilot experiment raises unexpected new questions, a subsequent pilot experiment will be performed. The pilot studies and data gained from mouse experiments carried out over the last 5 years have allowed us to accurately carry out sample size calculations.

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

The project workflow has been designed in a stepwise manner to ensure that at each stage that a biologically significant effect is likely. We will use a combination of taking organs or tissues from a mouse and using it in the laboratory (ex vivo) and only performing experiments in genetically altered mice or giving mice drugs, where there is evidence in the previous steps ex vivo that there is an important effect to be investigated. The aim of this is to reduce the number of animals required during the project. Pilot studies with a minimum number of animals will be carried out in order to assess the validity of each experiment and to refine group size estimates. The pilot studies themselves will be

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based on the size effect obtained in the ex vivo assays. For Protocol 10 we will use any surplus animals, for example from the 3Rs enquiries list.

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

The methods used will generate the greatest amount of data from the minimum number of animals required to achieve our scientific objectives, whilst minimising any pain, suffering, distress or lasting harm. We routinely expect to gain multiple data sets from a single animal, for example conducting multiple platelet functional assays all from a single blood sample from a single mouse. We can do this because of the use of modern methods we use, such as flow cytometry analysis which we use extensively, that allows us to use very small sample sizes. The overlap in expertise between different members of staff who are trained to carry out the mouse experiments as well as the ex vivo experiments and analysis allows us to blind the experiments, whereby the person who carries out the ex vivo experiments and analysis is blinded to the actual identity of mice from which the samples are generated. It also allows several people to work in parallel in different tissues such as bone marrow and blood that need to be handled fresh, thereby maximising the data obtained from single animals.

A retrospective assessment of reduction will be due by 17 April 2028

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.

This project will use a variety of mouse models:

Analysis of blood cell function - The injections and superficial vein bleeds will only cause minor and transient pain. Mice will be surgically anaesthetised when terminally bled (exsanguinated) and therefore will not feel pain or suffering.

Haemostasis model -The injections and superficial vein bleeds will only cause minor and transient pain. Mice will be terminally surgically anaesthetised when the tail tip is cut and therefore will not feel pain or suffering as they will not regain consciousness.

Myeloablation (irradiation) - The injections and superficial vein bleeds will only cause minor and transient pain. Myeloablation will be administered at the lowest possible split dose. Following myeloablation animals will be allowed to recover for a minimum of four days before any further procedures occur. During this time the animal's behaviour will be closely monitored and weight measured a minimum of daily.

Transfused mice - The injections of terminally differentiated cells will only happen once and will only cause minor and transient pain.

Pulsed laser microscopy (use of a laser to cause injury) - Mice will be terminally surgically anaesthetised when undergoing pulsed laser microscopy and killed by a schedule 1 method and therefore will not feel pain or suffering as they will not regain consciousness.

Splenectomy (removal of the spleen) - Mice will be subject to general anaesthesia during the surgical removal of the spleen. The wound will then be closed, pain relief will be administered, and the animal will then be allowed to recover from the anaesthesia. Mice will be housed in a warm cage for close monitoring during recovery. During the initial period of recovery from operation the animals will be checked every 10 min, then every hour until they can move freely when lightly disturbed. During this period and in the period post-surgery up to 2 months, endpoints will be monitored. At the end of the protocol, mice will be humanely killed with Schedule 1 method.

Myocardial infarction (MI) - Mice will be surgically anaesthetised and will be mechanically ventilated. The thorax (chest) will be opened and the arteries supplying the heart will be tied off to induce an MI. The wound will then be closed, and pain relief will be administered. The mice then be allowed to recover from the anaesthesia. Mice will be housed in a warm cage for close monitoring during recovery. During the initial period of recovery from surgery the animals will be checked every 10 min, then every hour until they can move freely when lightly disturbed. Animal heart function may be measured with echocardiography during this recovery period. Mice will receive a transfusion of cells via the tail vein post MI. During this period and in the period post-surgery up to 2 months, endpoints will be monitored. In terms of general endpoints, animals showing clear signs of social distress will be killed by a Schedule 1 method. Animals that have a loss of body weight of 20% relative to the baseline will be killed by a Schedule 1 method. Animals will be monitored for distress, reduced mobility, breathing abnormality or uncontrollable bleeding. We will kill mice using a humane method if they display any of the following specific endpoints:

(i) Heart Failure: Any animals showing symptoms and/or body weight loss equal to or greater than 20%, or echocardiography readouts showing 50% or greater reduction in cardiac output relative to baseline

(ii) Post-surgery pain: Any animals showing signs of pain that are not controlled by pain relief

(iii) Bleeding during surgery: Any animals in which bleeding cannot be stopped

(iv) Reopening of the surgical site: Animals with a reopening after the first instance

(v) Surgical site infection: If no improvement is observed within two days of treatment

At the end of the protocol, mice will be humanely killed with Schedule 1 method.

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

Mice are the species of choice for the proposed investigations because they are a good mammalian model with well-characterised haematopoietic (blood) system and, in particular, megakaryocytes and platelets that are very similar to humans. There has been very little work done on platelet function in non-mammalian species. The mouse is the species in which reliable transgene technology is best established. Mouse model of transfusion are also well-established and provide safety and functional data recognized by the regulatory bodies for applications for human trials.

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

To generate transgenic mice (mice with altered genes), wherever possible we will use mice where we can turn on the mutation, rather than having the mutation from birth. This means that we should not see the effects of this mutation until we turn it on. We will only use well-established reagents and protocols to turn on or off the candidate gene. The effects of the mutation will also be limited to the cells of interest using, whenever possible, specific deletion of the gene of interest in the blood system or in cells from which megakaryocytes are derived. When exposing mice to irradiation this may be administered as a split dose to give improved recovery rates.

For the myocardial infarction (heart attack) model and for splenectomy (removal of the spleen) we will use a comprehensive set of clinical sign assessments, humane endpoints, for animals that have undergone surgery. In summary, animals showing clear signs of social distress will be killed by a Schedule 1 method. Animals will be monitored for distress, reduced mobility, breathing abnormality or uncontrollable bleeding, and scored as per a tabulated list based on appearance, clinical signs, unprovoked behaviour and behavioural responses to external stimuli. For a total score that indicates moderate changes animals will be monitored daily, and NVS may be consulted. For a total score that indicates significant changes animals will be monitored closely and be prepared to euthanise the animal. NVS will be consulted. Any animal scoring maximum in any given category, or a maximum total score, will be immediately euthanised.

Clinical score sheet

Appearance

Score= 0. Normal, coat is smooth, lies flat and often has a sheen, eyes are clear and bright.

Score= 1. Slightly ruffled coat but no other marked changes

Score= 2. Moderate ruffled coat, eyes and nose may have discharges

Score= 3. Very ruffled coat, external orifices ungroomed, abnormal posture, eyes look pale,

pupils enlarged.

NB Check for normal or non-normal urine and faeces

Clinical Signs

0. Respiration appears normal, body temperature feels normal on handling, no twitching

behaviour, normal bowel movements.

1. Small changes in above parameters. Weight loss <10% of controls. Up to one toe

necrosis (B-D).

2. Body temperature above normal, respiration rapid and shallow, twitching behaviour,

altered bowel movements. Weight loss 10-20% of control animals.

3. Marked increase in body temperature, respiration noisy, comatose OR Weight loss >20%. Infection at suture site.

Unprovoked behaviour

This behaviour is best observed from a distance and before any handling is attempted.

0. Normal behaviour pattern

1. Minor changes, e.g. slightly altered walking pattern (WP).

2. Abnormal behaviour, decreased mobility and alertness, inactive. Resolvable dragging of hind legs.

3. Unsolicited vocalisation, self-mutilation, expiratory grunts, very restless or does not move at all

Behavioral responses to external stimuli

Often animals will show inquisitiveness with whisker twitching and sniffing or attempts to escape if frightened. Animals can have good body tone on handling. If the abdominal area of the body is painful then gently pressure and observation is a useful measure to pain.

0. Behavioural responses normal for the expected conditions

1. Shows some minor depression or minor exaggeration of responses.

2. Shows moderate signs of abnormal responses, there may be a change of behaviour.

3. Reacts violently to stimuli or muscular responses may be very weak as in a pre-comatose state

Scores are added for each of the categories above (appearance, clinical signs, unprovoked behaviour, behavioural responses to external stimuli).

For Total Scores 0-2, no action need be taken.

A Total Score of 3-5 indicates moderate changes, which will be monitored daily, and NVS may be consulted.

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A Total Score of 6 or 7 indicates significant changes, which will be monitored closely and be prepared to euthanise the animal. NVS will be consulted.

Any animal with a score of 3 in any one of the categories in the tabulated list, or a total score of 8 or above, will be euthanised.

Where possible we will use refinements in husbandry, for example animals will be group housed and extra enrichment will be given to those strains exhibiting aggression. Extra enrichment and a mashed diet will also be given to any animals exhibiting signs of inappetence (not having an appetite) based on weight loss. Additionally we will use less scary handling techniques where possible, and always allow acclimatisation of the animals when brought into the facility before starting a study.

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

- We will follow the ARRIVE and LASA guidelines.
- We will follow the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines.
- The humane endpoints webinar (https://www.humane-endpoints.info/en).
- The NC3rs resources for breeding and colony management in genetically altered mouse colonies (https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management).

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

- We will routinely check the NC3R's resource library and keep abreast with, and implement where appropriate, any new advances in refinement, reduction and replacement.
- We will have regular discussions with the Named Persons and animal technicians within the facility to review current approaches and whether there are any new 3Rs opportunities.
- We are subscribed to an internal 3Rs enquiry list in order to keep up to date with 3Rs news and events, as well as opportunities to share tissues and knowledge.
- We will regularly check an internal website which has a wide variety of resources and information including a 3Rs search tool.
- We will subscribe to publications such as ATLA (Alternatives to Laboratory Animals) Journal.
- We will attend NC3Rs events and workshops where appropriate.

A retrospective assessment of refinement will be due by 17 April 2028

The PPL holder will be required to disclose:

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- 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?

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