



Home Office

## NON-TECHNICAL SUMMARY

# Therapeutic Potential of Tregs

### 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

T regulatory cells, Therapy, Transplantation, Autoimmunity

### Animal types

### Life stages

Mice

adult, neonate, juvenile, embryo, pregnant

## 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 overall aim of this project is to explore novel strategies for increasing T regulatory cell numbers. T regulatory cells (or "Tregs" for short) are immune cells that play a key role in keeping the immune system under control.

**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?**

It is important to undertake this work as there are many instances where increasing Treg numbers would be beneficial for patients.

For example, increasing Tregs may help prevent the rejection of transplanted organs. Organ rejection occurs when the recipient's immune system "sees" the donor organ as foreign, triggering a response that ultimately destroys the transplant. Currently patients receiving organ transplants are treated with immunosuppressive drugs which reduce the risk of rejection by dampening down their overall immune response. However these drugs are not fully effective (despite these drugs a significant proportion of patients still reject their transplant), and they have side-effects, including leaving patients more susceptible to infections. Increasing Treg numbers may: (i) reduce the risk of rejection further and/or (ii) allow the dose/duration of immunosuppressant treatment to be reduced, so reducing side effects.

Increasing Treg numbers may also be helpful in treating patients with autoimmune diseases - such as multiple sclerosis. Autoimmune diseases occur when a patient's own immune system mistakenly "sees" their own tissues as foreign, damaging them. In multiple sclerosis it is the coating of the nerves in the brain and spinal cord that is attacked and damaged leading to disability; this coating is called myelin. Dampening down an over-reactive immune system by boosting the number of Tregs may be a safe therapy to try.

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

We expect to see several outputs at the end of this project. Including:

1. Generating new information. In particular we will determine if we can increase Treg numbers within our animals using two strategies (a) by transplanting laboratory expanded (outside the body) Tregs into our animals; (b) by giving a drug called IL-2.
2. Publications and presentations. We expect to publish the results of these studies in peer-reviewed scientific journals. We also expect to present the findings of our work at local, national and international scientific meetings.
3. Intellectual property that may support a planned patent application. We have recently identified a novel type of Treg, present in the blood and tissues of humans. In addition to testing "standard" Tregs in our animal models, we will also test these "new Tregs" in this project. We hope this will generate additional data to support our planned patent application.

4. Data to support future funding applications/clinical trials. We are very excited about the potential therapeutic applications of our work. And we hope that data generated in this project will help us apply for additional funding to take this work forward and "nearer to the clinic".

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

Beneficiaries of this work will include:

1. In the short-term - our work will benefit the clinical and scientific community working on Tregs, including individuals working on strategies to increase Tregs within the body.
2. In the medium to longer-term - if we demonstrate that IL-2 increases Treg numbers in one of our animal models (which will mimic what happens to the immune system of patients with multiple sclerosis (MS) receiving treatment with an immune-depleting drug called alemtuzumab) we will carry out a clinical trial of IL-2 in MS patients treated with alemtuzumab. Alemtuzumab is a highly effective treatment of MS, but it has side-effects. In particular, 50% of patients treated with alemtuzumab develop thyroid autoimmunity - that is, after treatment, their immune system starts to attack and damage their thyroid gland (instead of damaging the protective coating of the nerves). We believe we can reduce the risk of this happening by boosting Treg numbers with IL-2. If we are correct our work will significantly benefit individuals with MS, allowing them to receive treatment with a highly effective drug, without the risk of complications.
3. In the long-term our work on laboratory-expanded Tregs and our "new type of Treg" has the potential to help patients undergoing organ transplantation, and also patients who suffer from autoimmune conditions such as MS.

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

We will maximise the outputs of our work by:

1. Collaborating with other groups - including groups working on therapies for MS, and groups working on understanding Tregs. We have a strong collaboration record.
2. Disseminating new knowledge - through presenting our work at local, national and international meetings and by publishing our work in peer reviewed journals. We will also aim to publish any novel protocols we develop, and negative data/unsuccessful approaches.

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

- Mice: 2,250

## **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.**

1) We will use adult mice that lack an immune system of their own (such as NOD-scid IL2Rgamma<sup>null</sup> - also known as NSGs) to answer objective 1 *"Are expanded novel human Tregs suppressive in vivo?"* Because these mice lack an immune system, they can be "humanised". That is - when human immune cells are transplanted into the mice (by injection), they can survive and form a working immune system. This process is called engraftment. We have extensive experience of working with NSGs and know that they are the best type of mice to use for these sorts of experiments.

2) To answer objective 2 *"Does low dose IL-2 increase Tregs following lymphocyte depletion?"* we will use adult human CD52 "transgenic" mice (hCD52tg) or wild type mice. hCD52tgs have been genetically engineered to express a human version of a molecule called CD52 on their immune cells. This allows us to mimic what happens to the immune system when patients with multiple sclerosis are treated with a drug called alemtuzumab. Alemtuzumab binds to, and depletes, cells expressing CD52 (immune depletion). After treating the mice with alemtuzumab we will see if we can increase Treg numbers by giving the mice IL-2. hCD52tg mice have previously been shown to closely mirror immune depletion and immune recovery seen in humans following treatment with alemtuzumab, so are the appropriate animals to use to address this question. In this project we will also study the effect of low-dose IL-2 in promoting Tregs following other immune-depleting drugs, including cladribine another treatment licensed for multiple sclerosis. Wild-type animals or hCD52tgs can be used in these experiments.

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

To answer objective 1 *"Are expanded novel human Tregs suppressive in vivo?"* adult NSGs will receive a human skin graft. During this procedure a small piece of human skin (obtained with appropriate consent, typically from deceased organ donors or from patients undergoing cosmetic surgery) will be stitched onto the mouse's back after removing a very small piece of mouse skin. This will be done under a general anaesthetic and the mice will be given painkillers afterwards if needed. Once the skin graft has healed, the mice will be "humanised" by transferring human immune cells plus or minus Tregs intravenously (i.e into a vein) or intraperitoneally (i.e. into the body cavity). Prior to immune transfer the mice may be exposed to low dose irradiation, that makes 'space' for the incoming human immune cells to grow. Read out from this type of experiment is rejection of the skin graft (from time of humanisation). Transferring Tregs into the mice should delay or prevent graft rejection. Mice may be imaged to see where the Tregs have redistributed within the mice, if done this will be performed under a general anaesthetic.

For objective 2 *"Does low dose IL-2 increase Tregs following lymphocyte depletion?"* adult hCD52tg mice (and non transgenic litter mate/ wild-type controls) will be treated with alemtuzumab (or other immune-depleting drugs such as cladribine). Following immune depletion, the animals will be treated with low dose IL-2 or placebo (i.e. an inactive sterile liquid) given subcutaneously (i.e under the skin). The ability of low dose IL-2 to increase Treg numbers will be assessed by serial blood tests.

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

1) For objective 1 *"Are expanded human CD8 Tregs suppressive in vivo?"*

- All mice will undergo a brief surgical procedure under a general anaesthetic to receive a skin graft. Similar to humans, it is common for mice to be a little "groggy" for a short while post-anaesthesia, and for a day or so they may go off their food slightly. The mice are expected to recover well from the skin-grafting, and no adverse effects or only mild soreness at the graft site is anticipated.
- No or minimal adverse effects are expected from transferring a human immune system (humanisation) of these animals other than the transient discomfort from the injection.

2) For objective 2 *"Does low dose IL-2 increase Tregs following lymphocyte depletion?"*

- Treatment with alemtuzumab has been shown to cause either no or very mild effects when given to hCD52Tg mice.
- From the literature, we expect no or only very mild effects from treatment with cladribine.
- IL-2 is a naturally occurring chemical messenger (or "cytokine") and is not known to cause any adverse effects in treated mice when given at low doses.
- Administering alemtuzumab (and other immune-depleting drugs such as cladribine) and IL-2 will require the mice to have an injection which may cause transient discomfort at the injection site.

**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)?**

For objective 1 *"Are expanded novel human Tregs suppressive in vivo?"*

- Mild - 25%
- Moderate - 75%

For objective 2 *"Does low dose IL-2 increase Tregs following lymphocyte depletion?"*

- Mild - 90%
- Moderate - 10%

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

- Killed
- Used in other projects

## 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?**

We need to use animals to address objective 1 "*Are expanded novel human Tregs suppressive in vivo?*" because this is the only way to assess the full suppressive capability of these cells, including how they migrate into different tissues. Whilst experiments done in the laboratory in test tubes and petri dishes (*in vitro*) are useful, and will be used as much as possible throughout the project, they can not mimic cell to cell interactions that take place in a tissue/organ environment over time. For this, experiments in living animals (*in vivo*) are needed.

We need animals to address objective 2 "*Does low dose IL-2 increase Tregs following lymphocyte depletion?*" because it is not possible to mimic immune depletion and recovery over time in the laboratory. Furthermore, it is not possible to assess the behaviour of IL-2 within the body in a "petri dish".

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

There is no non-animal alternative to address objective 1 "*Are expanded novel human Tregs suppressive in vivo?*"

To address objective 2 "*Does low dose IL-2 increase Tregs following lymphocyte depletion?*" we did consider going straight to a small clinical trial in humans. We considered this because low dose IL-2 is being given to human patients in other clinical settings and is well tolerated. However, as described below, we concluded that this is not appropriate.

**Why were they not suitable?**

We did not think it was appropriate to go straight to a human study to address objective 2 "*Does low dose IL-2 increase Tregs following lymphocyte depletion?*", because, despite low dose IL-2 being safe in humans in other clinical settings, from our extensive *in vitro* work, we have concerns that it may cause problems after alemtuzumab or other such immune-depleting drugs.

Low dose IL-2 normally only expands Tregs. Other immune cells (called T-cells) also express the receptor for IL-2, so can respond to it. But their level of receptor expression is much lower than that of Tregs, so they are unable to respond to low doses. However, we have shown (through extensive *in vitro* studies) that expression increases after alemtuzumab (and the same may apply following treatment with other immune-depleting drugs). It is possible therefore, that low dose IL-2 might expand up unwanted aggressive T-cells (as well as Tregs) post-treatment. In patients this might lead to a worsening of their disease. It is therefore essential that we test this novel treatment strategy in mice first.

## 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?**

This number has been estimated from our extensive experience of doing this kind of work. We have estimated that 250 mice will be required for "rederiving" (i.e. establishing a new colony of mice from frozen eggs and/or sperm) any further strains of mice we need for this project. Up to 2000 mice will then be used to answer the experimental questions.

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

During the experimental design phase we (i) sought guidance from the NC3R's experimental design assistant on-line tool and (ii) reached out to collaborators who have extensive experience of the specific animal models we are using. In addition, we have in house experience of use of low dose IL-2 to expand Tregs *in vivo*, and our number estimates are based, in part, on these data. Our experimental design has also been informed by effect sizes seen *in vitro* (particularly for our work on response to IL-2 post-alemtuzumab).

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

To minimise animal usage we will:

- Breed animals as efficiently as possible - attempting throughout the protocol to match breeding with experimental need as closely as possible. This will require careful experimental planning.
- Throughout the project we will perform our experiments on small groups of mice (3-5 animals at a time), in pilot studies, and will review the results prior to extending our work to larger numbers/ we will adapt our work accordingly.
- Share animals with local collaborators - this is particularly relevant for NSGs where there are a number of groups locally using this animal model. This is something we routinely do on our current licence.

## Refinement

**Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.**

**Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.**

The two mouse strains that will be used during this project (i.e. NSGs and hCD52tg) are normal in appearance. The NSGs are more susceptible to developing infections and the hCD52tg will become more susceptible once treated with alemtuzumab - but this will be mitigated by keeping these animals in a near-sterile environment.

Animals used to answer objective 1 "*Are expanded novel human Tregs suppressive in vivo?*" are not expected to suffer any distress other than that caused at the time of the skin graft surgery. A transplant model is required to address objective 1, and skin grafting is the least invasive transplant model and the transplant model with least expected complications. The surgery is done under general anaesthesia and pain relief is administered. The mice are closely monitored for the few hours following surgery and until they fully regain consciousness, this normally occurs over the first 2-3 hours by which time they are usually behaving normally. Mice will then be followed closely over the next few days and weighed daily. We expect the mice to recover well from the surgery but will be watching for weight loss and any signs of over-grooming at the site of the skin graft. Mice will remain whenever possible in their social groups.

Animals used to answer objective 2 "*Does low dose IL-2 increase Tregs following lymphocyte depletion?*" are not expected to experience any harm, aside from the transient discomfort from the injection(s). We expect little in the way of any adverse effects from these experiments but will monitor the mice daily and keep constant weight measurements for each mouse.

**Why can't you use animals that are less sentient?**

We cannot use a "lower species" for our work, that is less sentient - because we need to study an animal with a similar immune system to that of humans, and therefore we need to study a mammal.

Furthermore, the two models we are planning to use in this project - engrafting of a human immune system, and transgenic expression of the human protein CD52 - have only been established in mice.

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

All animals used on this project will be monitored daily for any signs of ill health or distress. Particular care will be taken after skin-grafting, with increased frequency of observation over the first few hours post-operatively, and through the use of pain medication which if need be could be provided in the way of flavoured jelly (or such-like) that can be self-administered by the mouse. Immediately post-operatively mice will be kept warm and then will be housed in cages with additional soft bedding.

Throughout our project, we will aim to improve the quality of life for our animals – for example by providing enrichment, housing mice in social groups whenever possible and by avoiding single housing (apart from in exceptional circumstances, which will be time limited).

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

We are aware of, and will always aim to adhere to, good practice guidelines (as given by the Laboratory Animal Science Association; LASA). For example for objective 1 "*Are expanded novel human Tregs suppressive in vivo?*" we will ensure that we follow the LASA guidelines for performing aseptic surgery (as given in the updated 2017 document) – such as writing a pre-operative plan, administering pain medication and fluids as required and most importantly ensuring that the procedure is performed by a researcher who is fully trained.

We are also aware of the ARRIVE Guidelines (version 2.0), including their reporting guidelines around the importance of including information on experimental design and statistical analyses and strategies to minimise bias and how to report in publications.

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

We will remain informed about advances in the 3Rs by speaking to colleagues locally (including veterinary surgeons, senior animal house technicians etc) and by reading information sent to us by the local licencing department. We will also ensure that we stay up to date by visiting relevant websites (including that of the NC3Rs and Norecopa) and by attending relevant conferences and training events.

As a project team we will meet regularly to ensure that we are adhering to the 3R guidelines, and implementing any changes, effectively.