HOME OFFICE LICENSING

Since the last meeting, the ERP Certificate Holder's Advisory Group has recommended three applications for amendments to existing project licences and two applications for continuation project licences.

The Committee is asked **to receive and note** the following:

Applications

Project Title: **The calcium-sensing receptor in the cardiovascular, renal and respiratory systems**

An application to continue work from an existing Project Licence. The application has been granted by the Home Office, and the project abstract is reproduced below for further information.

Project Title: Impact of feeding patterns on endocrine metabolism

An application to continue work from an existing Project Licence. The application has been granted by the Home Office, and the project abstract is reproduced below for further information.

Amendments:

Project Title: The genetic control of cancer

An application to amend an existing Project Licence. The application has completed ERP and is currently with the Home Office, and the lay summary is reproduced below for further information.

Project Title: Strategies for Brain Repair
A fast track application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

Project Title: Induction of Anti-Tumour Immunity
An application to amend an existing Project Licence. The application has completed ERP and is being prepared for Home Office submission, and the lay summary is reproduced below for further information.

Abstract of the above appear below.

BSO
10 Oct 2012

LAY SUMMARIES/ABSTRACTS FROM APPLICATIONS PROCESSED BY CHAG

Please **NOTE** – these are for information only – no assessment by the Committee is required

Application 1 – The calcium-sensing receptor in the cardiovascular, renal and respiratory systems

Abstract. Genetic and acquired disorders of mineral ion metabolism in humans have demonstrated that the extracellular calcium-sensing receptor, CaSR, plays an essential role in maintaining a stable calcium concentration in the blood. Our recent studies have shown that this receptor also regulates the development of several organs *in utero*, and that loss of CaSR expression is associated with cardiovascular disease. The purpose of this work is to use genetically altered mice to help understand the role of the CaSR in development more fully, and how loss of CaSR expression leads to adult pathological conditions such as cardiovascular, renal and respiratory disease.

The developing role of the CaSR will be investigated *in-vitro* in organs explanted at different stages of mouse development. Parallel studies carried out in human foetal lungs and human adult blood vessels carried out in our laboratory have shown the validity of the mouse model for these studies. However, genetic manipulation of human foetal tissue is clearly impossible, and the effects of long-term CaSR ablation on cardiovascular, renal and respiratory diseases cannot be fully investigated in humans, hence the need to use murine models of CaSR ablation. Evidence gathered by us and others shows that the mouse model of CaSR ablation we plan to use under the renewal of this Project License recapitulates the phenotype of humans with inactivating mutations of this receptor, hence, the suitability of this model to human pathophysiology. All *in-vivo* studies will be carried out in adult mice and are non-invasive and/or of carried out in anaesthetised animals (*i.e.,* MRI followed by perfusion fixation for tissue collection). The *in vivo* studies will also include 24 hour urine/faeces collection, non-invasive measurements of blood pressure in conscious animals using the tail cuff system, blood sample collection from the tail vein and non-invasive plethysmography. Always, we will try to minimise stress and discomfort for the animals, for instance by applying a local anaesthetic for the blood sample collection. At the end of the *in vivo* studies, and to reduce inter-animal variability, tissue will be collected from our animals for *in vitro* and *ex vivo* studies. In all circumstances, tissue will be excised from our animals under deep terminal anaesthesia.

In order to ensure that we do not use any more animals than necessary, we have used a power calculation to determine the sample size. The CaSR is an important drug target and, indeed, activators of this receptor (or calcimimetics) are therapeutically used for the treatment of several disorders of mineral metabolism while antagonists are in phase III clinical trials in humans for the treatment of osteoporosis. These drugs are well tolerated and have a good safety profile.

Our studies will determine whether adult cardiovascular, renal and respiratory conditions arise from an altered Ca^{2+} homeostasis *in utero*. In addition, they might lead to the possibility of using calcimimetics to rectify an impaired foetal development or the possibility of using these drugs for cardiovascular disorders which arise as a consequence of kidney failure, such as hypertension, atherosclerosis and vascular calcification.

Application 2 – Impact of feeding patterns on endocrine metabolism

Abstract. This project we will determine whether the pattern of feeding has a significant impact on the development of obesity, fertility, neurodegenerative disease and Prader-Willi syndrome and will validate a novel probe for hormone detection.

The current obesity epidemic has occurred in the context of a shift away from meal feeding and towards grazing behaviour, but the impact of feeding patterns on mammalian physiology remains almost completely unexplored.

Our studies in rats indicate that grazing increases the efficiency of fat storage and elevates the secretion of the hunger hormone ghrelin. In this project we will establish the effects of grazing (and other patterns of food intake) on the choice of food selected, the efficiency of food absorption (including an assessment of gut bacterial function), metabolic hormone secretion and the way in which nutrients are stored in fat.

Our studies also indicate that meal-fed rats are able to maintain skeletal growth, despite reduced food intake and fat mass. Since these data suggest that manipulating the feeding pattern may promote healthy growth in conditions of malnutrition, we will establish the skeletal effects of varying the pattern of feeding with restricted food access.

In addition, we have also demonstrated that grazing shifts the balance of testicular function from testosterone secretion to sperm production, suggests that the pattern of feeding may have an impact on male fertility. Many of the variables of reproductive function will be assessed from tissue collected from studies on adiposity and growth, but we will also establish the effects of feeding patterns on reproductive hormone secretion.

We have shown that pre-treatment with ghrelin prevents degeneration of neurones in a standard rat model for Parkinson's Disease. We will therefore establish the mechanisms mediating this effect and determine whether neuroprotection may also be achieved by manipulating the pattern of feeding.

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder in which ghrelin secretion is profoundly increased. We will develop new genetically-altered models of this condition to determine which of the symptoms of PWS are dependent upon increased ghrelin.

Many hormones are secreted in bursts, necessitating the collection of sequences of blood samples over a prolonged period. To overcome the need for blood withdrawal, we will develop an *in vivo* probe enabling quantification of circulating hormones in conscious animals. This new tool represents a significant advance in refinement and reduction, enabling more rigourous analysis of hormone secretion with applications in clinical and veterinary medicine.

Thus, this moderate severity project will advance our understanding of the physiological consequences of feeding patterns. The pain, distress and lasting harm caused is expected to result in novel, cost-effective strategies for the prevention of obesity, the promotion of healthy skeletal growth in the developing world, slowing the decline in male fertility, slowing brain cell loss in Parkinson's Disease and alleviating the symptoms of Prader-Willi syndrome.

Amendment 1 -

Lay Summary: PPL title The genetic control of cancer

1. What the changes are:

New Protocol 12 to allow the development of tumour transplantation in genetically similar (syngeneic) mouse models of cancer

2. Why the changes are needed:

Genetically engineered mouse models of cancer develop sporadic and widespread tumours through out the organ(s) of interest. This makes any future experiments involving radiation (with and without novel anti-cancer agents) particularly difficult in terms of target location. Widespread total body irradiation is a poor substitute for therapeutic irradiation in solid tumours as it is poorly tolerated adversely affecting animal health and does not accurately reflect clinical practice. The ability to create a mouse allograft model where tumours are localised circumvents these issues and will permit studies incorporating targeted irradiation. This will move forward an important area of pre-clinical research in mouse models with an intact immune system.

3. Species and number to be used

The additional procedure of allograft transplantation in Protocol 12 is to be applied to 200 adult mice as continued use from existing protocols. Pilot studies will be undertaken initially to limit potential use.

4. Effects on the animals:

Tumour tissues from donor mice will be transplanted into recipient mice and as donor and recipient mice will have the same genetic background tumour rejection should be avoided. Mice will be anaesthetised during the procedure to transplant tissue (tumours cells in suspension or tumour tissue fragments) via injection or into a subcutaneous incision. Mice will be provided analgesia following injection to limit discomfort and closely monitored to ensure no unnecessary harm.

Tumour burden in animals will be limited to the minimum required for a scientifically valid outcome in keeping with published guidelines (Workman et al.). Efficacy studies will be terminated once durable, statistically significant therapeutic effects can be demonstrated. Any therapeutic studies will be designed to avoid the need for control tumours to become excessively large. Tumour growth will be established in the flank region(s). Assessment of superficial tumour size will be done using callipers of two diameters at right angles. Variation in measurement will be reduced by ensuring the same individual obtains measurements for the duration of a study and will be performed once weekly, or more frequently dependent upon growth rates. For animals carrying a single tumour, the diameter will not normally exceed 1.5cm. Where two tumours are grown, for example in the contra-lateral flanks, the size will be correspondingly reduced and not exceed the maximal tumour burden of a single tumour. Necrosis resulting in skin breakdown or exudation persisting beyond 48 hours will be grounds for termination. Mice will be monitored on a daily basis for clinical signs of ill health (e.g. weight loss, abnormal vocalisation, pilo-erection or hunching) or more frequently if clinical concern exists. Severe symptoms are unlikely

to occur and should be avoided, however, clinical signs necessitating immediate intervention (humane termination) will include:

i) Failure to eat or drink over a 24-48 hour period resulting in dehydration/emaciation

ii) Consistent or rapid body weight loss reaching 20% at any time or 15% maintained for 72 hours compared with pre-treatment weights

iii) Hind-limb paralysis or weakness

iv) Tumours that interfere with locomotion or cause abnormal vocalisation, animal behaviour or function.

In all cases the general health and condition of an animal will be the overriding concern.

5. Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

Recovery from anaesthesia. Limited duration discomfort (2 days)

b) Potential benefits:

This work will provide additional data to address the objectives of the PPL in terms of increasing our understanding of therapeutic control of cancer using irradiation and novel anti-cancer agents.

c) How the benefits outweigh the costs:

This new model will allow direct targeting of irradiation and thus prevent unnecessary more widespread irradiation of the mouse. This clearly will limit the side effects and suffering of animals. This will also allow a more accurate reflection of clinical practice, allowing the generation of data which can address important questions and reduce the need future animal experimentation. We will also be able to directly observe tumour response to therapy which is a distinct advantage when tumours are concealed deep within an organ,

This work will be done with animal welfare a top priority and involve the veterinary services available where appropriate to maximise animal well being

Workman, P., Aboagye, E.O., Balkwill, F., Balmain, A., Bruder, G., Chaplin, D.J., Double, J.A., Everitt, J., Farningham, D.A., Glennie, M.J., Kelland, L.R., Robinson, V., Stratford, I.J., Tozer, G.M., Watson, S., Wedge, S.R. & Eccles, S.A. Guidelines for the welfare and use of animals in cancer research. *Br J Cancer*, **102,** 1555-77.

Amendment 2 -

Lay Summary: PPL title: Strategies for Brain Repair

1. What the changes are:

One addition to 19b1:

1. To allow the preparation of a graft bed up to $1cm^3$ on host rats and mice and grafting of rat, mouse or human skin onto this area.

2. Why the changes are needed:

We wish to further explore the mechanisms that underlie the immune tolerance produced by the early peripheral administration of non-compatible allogeneic or xenogeneic tissues. Thus far the tolerance has only been challenged by relatively 'immune privileged' neural grafts (the brain is less 'visible' to the immune system than peripheral tissues) but it would be valuable to examine the response to a peripheral immune challenge. This will allow investigation of the extent of tolerance induced by the method and therefore how successfully it may be applied as a method of immune protection in preclinical studies of neural cell replacement. The easiest and least invasive peripheral grafting process is a skin graft. This may be done with human skin or rat/mouse tail skin. This is in line with objective 4, under 'Immunological issues', which includes experiments designed to define mechanisms of the tolerisation method as an improved immune protection protocol allowing the assessment of human cells for clinical transplantation in xenograft models. Tolerising is usually carried out with human tissue to assess the survival of human donor cells; therefore a skin graft of the same tissue type will be necessary. Comparison of the host reaction to skin grafts of human tissue and of rodent tissue will be necessary to ensure tolerance has been induced and allow effective neural graft survival, therefore both will be required.

3. Species and number to be used

Donor rats and mice are already included in the 4000 rats and 2500 mice included in the standard rat protocol on the licence for this protocol as tail skin for several animals can be obtained from a single donor animal. Similarly the experimental animals are already covered by the same license allocation.

As few animals are required to provide enough donor tissue for grafting, 2-5 will be used per experiment to graft approximately 10 host animals.

4. Effects on the animals:

1. Donor:

   The donor animals are sacrificed by terminal anaesthesia prior to removal of the tail skin so the process is not invasive for them.

2. Host:

   Host animals have a graft bed prepared which involved trimming the skin back to the vascular bed. The graft is sutured into place and animals are bandaged as necessary to prevent them from removing the graft before it has taken to the graft bed. This can be mildly

irritating for the animal. Temporary individual housing may be necessary if cage mates are removing bandages.

Rejection of skin grafts to the periphery is more likely but this is unlikely to cause any significant discomfort to the animal. Rejection is apparent by discolouration of the grafted tissue; therefore the endpoint of the experiment may be more clearly defined than with a neural graft.

5. Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

1. Very few rats or mice are required to act as donors for skin tissue; graft material can be obtained for several grafts from one host. We expect that 2-3 rats and 4-5 mice will be required per experiment to be killed via schedule 1 methods and their tail skin removed.

2. Host animals will be bandaged and may be singly housed for a few days which will prevent them removing the bandage and keep group numbers to a minimum. Any difficulties in bandaging animals will be overseen and advised by the NVS.

b) Potential benefits:

1. Tolerisation for neural grafting removes the need for daily cyclosporine administration by i.p. injection and the possible loss of animal (necessitating greater group size) through weakness induced by the immune suppression.

2. The tolerisation technique, currently only tested in neural transplantation may be a) better understood and b) more widely applicable if we can demonstrate how peripheral grafts are managed by the tolerised host.

c) How the benefits outweigh the costs:

Currently the only way to explore peripheral transplantation in the rat of allo- or xenograft techniques is through the use of cyclosporine to immunosuppress the animals. The administration of cyclosporine to rodents does not permit long-term experimentation due to health and welfare issues so experiments are curtailed. This necessitates more animals being used and the stress of daily immunosuppression injections. The tolerisation technique, now used regularly in our laboratory for neural grafts, may be useful to make the same savings in animal welfare and numbers if it can be applied to peripheral challenges. Furthermore, understanding the mechanism of tolerisation in greater detail may encourage its use by others, and may provide valuable insights into the mechanisms of immune system function and organ rejection.

<u>Amendment 3 -</u> Induction of Anti-Tumour Immunity

1. Lay Summary: What the changes are:

Additional protocols 19b 3 and 4 to allow use of a model of leukaemia to be incorporated into the study (protocol 3) and to allow breeding of genetically modified mice (protocol 4).

2. Why the changes are needed:

Breeding: previously breeding was carried out under a licence held by s38 Health and Safety This licence expires in 2014.

Leukaemia model: To date we have examined the role of the immune system in limiting progression of solid cancers. It is likely based on published data that the type of immune manipulation under study will also be applicable to cancers of haematological origin. A haematologist has recently joined our laboratory providing us with the opportunity to expand the range of tumours under study in our lab to those of haematological origin.

3. Species and number to be used

600 additional adult mice in total to be used in the protocol 3 over the next 3 years of the licence

Up to 5000 mice will be bred (protocol 4) over the remaining 4 years of this licence. [Note – these are not additional animals; they would previously have been bred on the service licence]

4. Effects on the animals:

In order to induce leukaemia in mice, adult mice will be irradiated and subsequently injected with bone marrow cells, infected with retrovirus constructs in vitro. These infected cells form acute myeloid leukaemia in the recipient mice.

Animals will therefore be restrained for irradiation and subsequently for injection with bone marrow cells. At various time-points thereafter animals will be restrained for tumour monitoring purposes (blood sampling, imaging under anaesthetic). Each procedure will cause only transient discomfort to the animal. Use of a bioluminescence imaging system will facilitate detection of malignancy in animals prior to the onset of symptoms. However animals will be monitored daily for symptoms (major symptom listlessness, weight loss) during the period post bone marrow transplant.

5. Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

Up to 600 mice will be irradiated and receive bone marrow from donor animals killed via a Schedule 1 method. This will involve transient discomfort during the period of irradiation and injection. Malignancy in these animals can cause listlessness and weight loss. Animals will be monitored for these symptoms, which will be minimised as far as possible through detection of tumour growth by blood sampling and / or bioluminescence imaging. With these monitoring procedures in place, the main cost in terms of animal welfare is that the mice will have to undergo

restraint for the purpose of blood sampling and general anaesthetic and restraint for the purpose of imaging.

b) Potential benefits:

Currently, treatment of acute myeloid leukaemia involves the use of non-specific chemotherapy; a treatment often associated with significant toxicity and side-effects detrimental to patient health. In addition, despite initial remission, relapse will occur in over 50% of patients. For these reasons, development of immunotherapeutic strategies for the treatment of leukaemia poses an attractive alternative. The work proposed by the changes introduced in this licence will provide an additional model system through which the impact of the immune system on progression of leukaemia can be assessed.

c) How the benefits outweigh the costs:

The type of immune effector cells / molecules capable of controlling tumour growth *in vivo* have not yet been definitively identified. In addition, whilst many pathways of tumour-induced immunosuppression are thought to contribute to the overall paucity of anti-tumour immunity, these pathways remain poorly characterised. Work carried out by several laboratories including our own has revealed that a subpopulation of white cells, named regulatory T cells (Tregs) may play a key role in inhibiting effective anti-tumour immunity. In addition, we have also found that complement components drive cancer progression.

A two-pronged approach is now warranted to find out how this information can be harnessed for the purposes of treating people with cancer. Firstly, the immune responses capable of promoting tumour destruction must be identified and secondly the precise way in which regulatory T cells and / or complement act to stop these responses working effectively must be discovered. At present however, there are no methods available for replicating an intact immune system using laboratory-based methods. The reason for this is that the immune system comprises a complex network of different cell-types that interact within organised structures (lymphoid organs) in the body. Different components of the immune system may behave differently once removed from this environment. It is essential therefore that the effect of a part of the immune system is tested when these interactions are intact. The animal model to be used is a mouse model as over the past few decades, a large body of information has been gathered about the mouse immune system. Much of this information has shown that immune responses in mice closely parallel those in humans.

To date, our experiments have focussed on injection of cell-lines or carcinogens that result in development of solid tumours. A typical experiment would involve injecting a mouse with cell line / carcinogen and determining which components of the immune system are essential (or counter-productive) for allowing the mouse to mount an adequate immune response to the tumour. An adequate immune response is considered a response that controls or even halts tumour growth. Our work using these types of mouse models has already resulted in establishment of a clinical trial aimed at examining the impact of depleting Treg cells in patients with inoperable colorectal cancer (http://medicine.cardiff.ac.uk/news/colorectal-cancer-trial/).

We now wish to expand upon use of solid tumours to determine how the immune system can be manipulated for the treatment of cancers of haematological origin. As described above, currently, treatment of acute myeloid leukaemia involves the use of non-specific chemotherapy; a treatment often associated with significant toxicity and side-effects detrimental to patient health. In addition, despite initial remission, relapse will occur in over 50% of patients. For these reasons, development of immunotherapeutic strategies for the treatment of leukaemia poses an attractive alternative. The work proposed by the changes introduced in this licence will provide an additional model system through which the impact of the immune system on progression of leukaemia can be assessed.

Minimising adverse effects:

As described above, every effort will be made to minimise adverse effects through good handling techniques and careful monitoring of tumour growth.