Date of second strand review: 26 March 2014

Project title	**Development and refinement of Small Animal Imaging**		
Key words	Imaging, Development, Biomarkers		
Expected duration of the project (years)	5		
Purpose of the project	Basic research	Yes	
	Translational and applied research		No
	Regulatory use and routine production		No
	Protection of the natural environment in the interests of the health or welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals		No
Objectives of the project	This project aims to refine existing small animal (rats and mice) imaging techniques and develop novel imaging techniques and probes to be used by other P.I's and Project Licence holders. PET is a medical imaging technology that uses short lived radionuclides attached to biologically active molecules to produce images of metabolic processes in the body e.g. glucose metabolism. PET can be used to visualise abnormalities present in diseases such as cancer and neurological disorders (e.g. stroke). Magnetic Resonance Imaging or MRI provides information on structural and anatomical changes within a living organism. It can be used to assess the progression of disease such as Alzheimer's or brain tumours. The agents used to visualise disease or enhance anatomical structures need to be developed and refined to ensure they have relevance to the disease/ anatomical change being interrogated. Emerging imaging techniques will also be validated for routine use in the imaging laboratories. Imaging biomarkers are important for diagnosing disease, monitoring its progression, tracking response to therapy and enhancing knowledge of physiology. We need to understand the changes in imaging biomarkers during an intervention to ensure that we interpret data correctly		

	Such methods minimise the number of animals required in preclinical research since each animal can be scanned serially over time and each animal can be used as its own control (e.g. baseline scans). This avoids the need for killing groups of animals at fixed time intervals. These imaging techniques fully utilise the 3R's philosophy. In a majority of studies and where possible preliminary work will have been carried out using in vitro cell culture methods (PET) or in cadavers or phantoms (MR).
Potential benefits likely to derive from this project	This project will increase our understanding of imaging readouts and will allow continued refinement of animal imaging protocols and will help overcome the challenges common to all imaging techniques including (a) the design of probes or probing techniques that are specific to the biological process of interest, (b) optimization of imaging systems to provide the highest sensitivity and image resolution, (c) minimisation of perturbation to the biological processes under observation so that the experimental outcomes correlate to the biology and not the probing d) quantitation of the imaging signal and correlation to an underlying biological parameter. Non-invasive imaging offers a substantial refinement enabling repeat scanning of the same animal and subsequent reduction in numbers. Investigation of Imaging Biomarkers in response to a therapeutic intervention may help us to understand the changes in biology and could ultimately lead to the identification of clinically relevant biomarkers could potentially aid patient selection and disease monitoring which can have considerable benefit for patients, in terms of preventing the need for unnecessary treatments or allowing changes in treatment as soon as one is identified as "failing" and in terms of financial costs.
Species and approximate numbers of animals expected to be used, and anticipated period of time	Rats 600, Mice 900 over 5 years
Expected adverse effects and the likely/expected level of severity. What will happen	Many experiments will be executed under terminal anaesthesia and therefore the only adverse effect expected is anaesthetic overdose which is controlled by monitoring the animal during prolonged periods of anaesthesia.

to the animals at the end.	Generation of localised tumours may introduce infection at the site or introduce mouse pathogens although this is very rare. Aseptic conditions are used and new cell lines are screen for mouse pathogens before implantation. Tumour growth is occasionally associated with a reddening of the skin in superficial tumours. Burden is restricted to below 1.25cm3 that equates to less than 5% body volume. For new xenograft models or more complex disease models, Initial pilot studies will have been initiated on other PPL's to determine model progression/prognosis and to identify early indicators of a decline in well-being that could be used as an end-point indicator in subsequent studies on this PPL (for example general loss of condition or weight that precedes a severe adverse event). Imaging is used at early time points in disease progression prior to any detrimental effects as a consequence of disease burden. Imaging is also incorporated, if possible, where tumours are not palpable (brain tumours) to monitor disease progression. Other adverse events that could occur are: Treatments used can cause weight-loss and in superficial tumours, skin reddening or scabbing at the tumour site. Ulceration is very rare and would indicate mouse cull. Gaseous contrast agents may induce hypoxia but these will be well characterised and previously used in animal and/or human studies. Radiotherapy can cause adverse effects such as inflammation and tissue damage at high doses but on this PPL high dose will only be used with short imaging time points. e.g. <24 hours post treatment and tissue not intended for exposure will be shielded. Urinary catheterisation may cause infection but aseptic conditions and lubrication will be used and signs of discomfort will indicate a cull. Blood sampling may lead to loss of blood via sampling site but this can be avoided by good technique and blood loss can be prevented by local pressure or cautery. Stress is also a potential adverse effect when moving animals between sites and performing procedures without anaesthesia. In these cases Standard Procedures are adhered to and animals are assessed to ensure their 'fitness to travel'. In the case of procedures without

	anaesthesia, periods of restraint training are given to acclimatise the animal to the procedure. At the end of a series of regulated procedures the animals will be humanely killed by either a schedule 1 method or using a recognised method to collect blood or preserve tissue for histological analysis.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	Many of our objectives can be achieved in phantoms, fruits and vegetables, or cadavers, or in humans. Some of our objectives can be achieved in human volunteers or patients, but in many cases, in accordance with the declaration of Helsinki, it is unethical to perform human studies before suitable animal studies have been performed. Physiologic function (heart beating, lung and diaphragm movement) is required to develop/refine animal imaging protocols as physiologic function itself can have a detrimental effect on the imaging readout. Other non-animal techniques such as Liquid chromatography/mass spectrometry (LC/MS), combined with commercially available hepatocytes, has become an indispensable tool in evaluating the presence of these metabolites in target tissues, however some tracers do not produce good metabolites *in vitro* and *in vivo* studies must be used.
2. Reduction How the use of minimum numbers of animals will be assured	Imaging can use each animal as own control, allowing paired comparisons, thus increasing statistical power of experiments compared with terminal designs. Imaging studies are inherently sequential (only one animal can be scanned at any one time), so lend themselves to adaptive designs, which use fewer animals to achieve the same statistical power as conventional designs. Tumour Studies will utilise tumour models that have robust, consistent growth characteristics. The implant of cohorts for the imaging study will be staggered over time, with generally 4 mice imaged per day depending on tracer. This is repeated until the experimental group sizes are achieved. Archived imaging data is used to refine sample size calculations enabling a reduction in group sizes.
3. Refinement	Rats and mice are essential mammalian species as they

Reasons for the choice of species and why the animal model(s) to be used are the most refined, having regard to the objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	cover the great majority of well characterised disease models, they are well understood which means that results are likely to be interpretable. They will be used as they represent the species with the lowest neurophysiologic sensitivity and the protocol will be one that causes the least pain, suffering, distress or lasting harm and that the results could not be achieved by any other reasonably practical method not using protected animals. Imaging allows the measurement of small morphological changes in animals that may be clinically normal. This provides a refinement in comparison with clinical endpoints in clinically abnormal animals. Even where animals are clinically abnormal, it is often possible to use milder disease than with other assessments. Where possible, mice will be used in preference to rats. Where possible, normal animals will be used in preference to tumour-bearing animals. Where possible, terminally-anaesthetised animals will be used.