7 December 2015

Project title	**Precursor cell therapies for kidney diseases**		
Key words	Kidney Cell Mouse Therapy Scanning		
Expected duration of the project (years)	Five years		
Purpose of the project	Basic research	Yes	
	Translational and applied research	Yes	
	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	We will assess the potential therapeutic effects of precursor cells injected into mice with experimental kidney diseases. Our current studies show that: a. we have mouse models which mimic human kidney disease; b. we have precursor cells ready for testing as new biological therapies for kidney disease; c. we have scans with which to monitor kidney disease and administered cells in living animals. In this project, we will bring these lines of work together to determine both where cells go after they have been administered to mice with experimental kidney disease, and whether administration of such cells prevent kidney damage. These studies will pave the way for the human trails evaluating human kidney precursor cell therapy.		
Potential benefits	In the UK, 57,000 people have such severe kidney		

likely to derive from this project	disease that they require long term dialysis or kidney transplantation. Unfortunately, being on long term dialysis confers a high risk of death, exceeding that found in people who have certain cancers, and there are insufficient numbers of donors available to rescue all dialysis patients by kidney transplantation. Currently available drugs are only partially effective at treating kidney diseases. So, there is an urgent need to define new treatments to prevent people with diseased kidneys reaching a stage when they need dialysis. The current project, using mice with experimental kidney disease, is a step towards this end. Moreover, by using scanning, we will be able to track the fates of administered cells inside living animals, and these techniques may later be transferrable to humans (and also non-human animals) suffering from kidney diseases.
Species and approximate numbers of animals expected to be used, and anticipated period of time	Mice, including immunocompromised SCID mice. 650 over five years
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	The main part of the project has a 'Moderate' severity level. In a small part of this programme of work, however, we will optimise and refine models of kidney disease to be used as test beds for precursor cell therapy. Some of these mice may suffer weight loss and behaviour changes (e.g. poor grooming) after the induction of experimental kidney injury and this part of the programme would have a 'Severe' severity. At the end of each experiment the mouse will be killed by a humane method.
Application of the 3 Rs	
1. Replacement Why do animals need to be used,	We aim to determine whether the administration of precursor cells might be considered as a realistic therapeutic strategy for people with kidney disease.

and why non-animal alternatives cannot be used.	We, and others, have shown that such precursor cells can form kidney cells in culture. These experiments provide fascinating biological insights and are encouraging first steps towards therapies. However, neither normal kidney function nor the complex tissue changes of kidney disease, can be reproduced in cell culture. So, given the need for realistic preclinical models, there is currently no alternative to determining whether kidney precursor cells can treat kidney disease unless we use live animals. Moreover, the tracking of administered cells in whole animals is needed to gain insights into the mechanism of any beneficial effects they may be found to have.
2. Reduction How the use of minimum numbers of animals will be assured	The use of scans to monitor both the structure and function of kidneys, and also to locate labelled precursor cells which have been injected into mice with kidney disease, is a key feature of this project. While these techniques do require general anaesthesia, and sometimes also injection of chemicals, they are 'minimally invasive' and so can be used recurrently on a single mouse to monitor disease progression, obtaining a large amount and variety of data. This will reduce the total number of mice used in the study. The scanning results will be compared with kidney fibrosis found at autopsy. Such tissue analyses have previously been a 'gold standard' of mouse kidney experiments. In our preliminary experiments, we have quantified the proportion of the kidney occupied by scarred tissue. This allows us to calculate the minimum numbers of mice we would need to use to demonstrate a statistically significant and biologically important therapeutic effect.
3. Refinement Reasons for the choice of species and why the animal model(s) to be used are the most refined, having regard to the	At present, the mouse represents the best species with which to test the efficacy of precursor cell therapies. Progressive kidney disease which mimics human kidney disease has not yet been modelled in lower species such as fish and frogs. The mouse has a kidney of similar structure and anatomical complexity to humans. Moreover, mouse

objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	disease models can reproduce the anatomic and functional changes that occur in human kidney disease. Mice will be closely monitored (twice a day soon after the induction of kidney damage, and then daily during the 'chronic' phase of kidney disease. Particular attention will be paid to their weights and behaviour. Should these parameters deviate markedly from normal, mice will be humanely killed. Such effects are much more likely to occur in the 'dose finding' study in which we will optimise and refine models of kidney disease which will then be used as test beds for precursor cell therapy. The dose finding study only represents a small part of the total programme of work.