16 March 2015

Project title	**Proteolytic cleavage of the LDL receptor**		
Key words	Cholesterol, Low density lipoprotein receptor (LDLR), protease, inhibitor		
Expected duration of the project (years)	5		
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	Yes	
Objectives of the project	Cardiovascular disease kills one in three people in the UK. One of the most significant risk factors for cardiovascular disease is elevated levels of low-density lipoprotein (LDL) cholesterol – often referred to as 'bad cholesterol' – in the blood. The amount of LDL- cholesterol in the blood is controlled by its uptake into liver cells. On the surface of the liver cells is a specific protein, the LDL receptor, which binds the LDL- cholesterol. The number of active LDL receptors on the surface of liver cells is the single most important factor in regulating the amount of LDL-cholesterol in the blood. We have identified that the LDL receptor is cleaved into two smaller fragments by an enzyme. These smaller fragments of the LDL receptor are unable to take up the LDL cholesterol into the cells. Decreased LDL uptake results in increased circulating LDL, which can lead to high cholesterol levels. The overall aim of this project is to test the hypothesis that cleavage of the human LDL receptor regulates LDL receptor function and hence plasma LDL cholesterol in vivo. We will use mouse models to determine whether regulation of the activity of the enzyme may be a novel mechanism to lower the concentration of LDL-cholesterol in the blood.		
Potential benefits likely to derive	Our research will lead to improved knowledge and an advanced understanding of the fundamental science		

from this project	underpinning regulation of LDLR and cholesterol metabolism. This understanding will provide an advantage to academic researchers worldwide seeking to develop novel drugs to lower plasma cholesterol. This could have a significant global impact by potentially enhancing the quality of life, health and well-being of individuals affected by high levels of LDL cholesterol. Our research also has potential in the development and commercialisation of novel therapeutic treatments.
Species and approximate numbers of animals expected to be used, and anticipated period of time	Mouse. 670 mice over a period of 5 years
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	*Severity category* Moderate: While the severity category of the surgical procedure is moderate, the severity category of the majority of the work contained within this application is mild. *Adverse effects* 1. Slight and transient pain during ear-notching required for genotyping of animals generated from conventional or cross-breeding of animals. 2. Weight gain and skin changes from feeding the animals on a Western diet. 3. Pain from the wound and/or disruption of intestinal function after the implantation of osmotic mini-pumps. Most animals undergoing any dietary or surgical interventions will be sacrificed under terminal anaesthesia. Remaining animals will either be kept alive for breeding and maintenance for continued use in this project or other relevant projects as authorised under this license or sacrificed under Schedule 1 method.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-	We have performed extensive alternative studies to confirm that cleavage of human LDLR reduces cellular LDL uptake. Having performed these extensive studies we believe that we have exhausted the current available

animal alternatives cannot be used.	systems and have significant data to warrant confirmation of our findings in an animal model. We now need to confirm the effects in vivo to provide proof of principle before embarking on exploring possible therapeutic targets of this pathway. We have been unable to identify an alternative to an animal model to allow us to verify our findings, however if any alternative became available during the course of the project, we would implement these into our studies.
2. Reduction How the use of minimum numbers of animals will be assured	We have ensured that we have the appropriate control and experimental groups to enable us to make appropriate conclusions from our experiments and have rationalised our experimental design to ensure that we have a specified programme of work with clearly defined objectives to limit the number of animals we require. In addition, we have also performed power calculations, using our cell-based work as an informative measure of effect size, to ensure that the study is appropriately powered to give meaningful, statistically relevant, data while ensuring that we use the minimum number of animals in our work.
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 objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	*Species* As murine LDLR is not cleaved it is necessary for us to use mice expressing human LDLR. This model is the most relevant model that currently exists that will allow us to test our hypothesis in vivo a crucial step in determining whether this mechanism is physiologically relevant for the treatment of high cholesterol. Any dietary modulations have been restricted to the shortest time period possible, while the surgical interventions have been designed carefully with the administration of sufficient and appropriate anaesthetics and analgesics so as to cause minimal suffering, distress or lasting harm to the animals. During surgery and recovery animals will be monitored closely to identify any signs of distress or harm.