14 January 2015

Project title	**The contribution of mast cells and their mediators in inflammatory skin diseases**		
Key words	Mast cells, psoriasis, atopic dermatitis, allergy		
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	The overall aim of the project is to investigate the role and mechanisms of a type of tissue resident cell, mast cells, in inflammatory skin diseases. Mast cells are mainly thought of in relation to allergy; however, new studies have highlighted their importance in inflammation. Currently there are no effective therapies to block mast cell involvement in inflammation. This project will investigate the factors which can block mast cell mediated inflammation in skin conditions (e.g. atopic dermatitis, psoriasis).		
Potential benefits likely to derive from this project	Mast cells contribute to both acute and chronic inflammatory skin diseases for which effective treatment is currently limited. A deeper understanding of mast cell function in these conditions promises to help us not only to optimize current treatments, but also to propose new therapeutic targets for the future.		
Species and approximate numbers of animals expected to be used, and anticipated period	Mice will be used for all experiments and we expect to use up to 2000 mice for Protocol 1, namely the breeding and maintenance of genetically modified mice, of which we currently have 5 strains, and up to 2000 mice for protocol 2, Murine models of skin inflammation: passive cutaneous anaphylaxis, atopic dermatitis, psoriasis. These		

of time	experiments will be carried out over the course of 5 years.
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	All experimental protocols will produce some degree of skin inflammation, with anticipated moderate severity. The animals will be sacrificed when the experimental protocol is completed and the research end points are achieved. However, if animal suffering is deemed to exceed the set severity limit, and suffering cannot be alleviated, the animals will be euthanized. Furthermore, if we realise that the research end point, for whatever reason, is unattainable then the animals will be euthanized, despite the experimental protocol not being complete.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	The skin is a complex organ, which unfortunately cannot be studied in isolation. Blood supply and neurological input play key roles in directing skin responses. Perfused and innervated human skin equivalents would be an ideal replacement as animal experimentation should be limited to the unavoidable, however at present these systems are still under development. Furthermore, primary mast cells generated from bone marrow cells of mice and human blood progenitors and the few cell lines (human and mouse) available do not fully mimic the maturity, plasticity and mediator repertoire found in tissue resident mast cells. In using a small number of mice to look at skin inflammation in situ we may be able to identify potential therapeutic interventions.
2. Reduction How the use of minimum numbers of animals will be assured	We will use professional statistical advice and always use the minimal number of animals, which allows for solid statistically relevant results (maximised power), but will allow for us to reach our scientific objectives successfully. We will mostly be using two-way ANOVA statistical tests for the analysis, and type I errors will be controlled by choosing the significance level of 5%, with the power level set between 80 and 90%. We will make use of pilot studies to assess efficacy/doses/set up optimisation prior to a full-scale study. We will collect and store tissue and cell samples which we will make available to other scientists in the field so that these specimens can be used for further experiments in the future without the unnecessary repeat of animal experiments.
3. Refinement Reasons for the choice of species and why the animal	Laboratory mice strains are susceptible to experimentally induced skin inflammation, which reflects human skin inflammatory conditions. Animal welfare will be a priority across this project and will be taken seriously by all

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.	members of the research group. We will monitor animals on procedures daily to ensure there are no unexpected or adverse reactions to the procedures they are subjected to. Any animal seen to be in discomfort or distress, which, upon taking advice from the facility vet, cannot be relieved, will be removed from the study and euthanized.