February 2016

<table>
<tr><td>Project title</td><td>Genetic analysis of tumour development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer, tumour, inflammation, therapy, imaging</td></tr>
<tr><td>Expected duration of the project (years)</td><td>5</td></tr>
<tr><td>Purpose of the project</td><td>
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<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[1]</td><td>Yes</td><td></td></tr>
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<tr><td>Objectives of the project</td><td>Cancer can start when a group of cells in the body gain the ability to divide in an uncontrolled manner, ultimately leading to the formation of a tumour (lump). However, it has recently been recognised that for tumours to grow and spread, they must develop a supportive environment, primarily through re-educating certain cells of the immune system, to develop blood supply and protection against toxic agents. Thus, a therapeutic strategy that attacks cancer cells, whilst concurrently neutralising immune cells, has become a very attractive option by which to advance cancer treatment. This approach is supported by encouraging results in clinical trials where blockers of certain immune</td></tr>
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[1] At least one additional purpose must be selected with this option.

	molecules have stabilised the disease of patients with advanced cancer. However, the benefit of blocking a single immune molecule may be brief because tumours produce multiple immune molecules. Therefore, we need to improve our understanding of how the different cell types in the tumour interact to develop more effective cancer treatments.
Potential benefits likely to derive from this project	The primary potential benefit relates to new knowledge about tumour initiation, development and resistance to therapies. The aim is to disseminate our findings through academic publications and oral presentations. This information is of interest to biological scientists. The secondary potential benefit relates to clinicians, in particular oncologists. New molecular targets may be identified, for which pharmaceutical products could be designed. Imaging modalities may be developed to facilitate early clinical evaluation. A reduction in invasive procedures to assess tumour biology in pre-clinical models of cancer is anticipated.
Species and approximate numbers of animals expected to be used, and anticipated period of time	6,000 mice
Expected adverse effects and the likely/expected level of severity. What will happen to the animals at the end.	This project will create, breed and maintain mice with appropriate genetic changes. For the majority of animals, we anticipate that these genetic alterations will have negligible adverse effects and be of mild severity. Some genetic modifications may result in abnormalities causing moderately severe suffering. These will be minimised by appropriate breeding and husbandry methods. Tumours will be induced by expression of disease relevant genes, repeated exposures to chemical carcinogens, or injection of tumour cells. These models are chosen based on comparable disease aetiology with humans. The overall health of mice bearing benign tumours is generally acceptable, unless their number or size becomes excessive leading to distress. Consequently, tumour burden that will not exceed 5% of the host animal's normal body weight should avoid undue discomfort.

	Animals displaying invasive and internal tumours will be monitored regularly to allow detection of progression of malignancy. In the majority of instances, termination by a humane method at defined ages will be required. Substances may be administered in the diet or drinking water, by direct application onto the skin or by experienced licensees via oral gavage, enteral or parenteral routes. These procedures should not result in more than temporary pain, suffering or distress. If required, some procedures will be performed under light general anaesthesia and/or pain relief. Mice will also be used to develop imaging strategies to evaluate tumour development and changes in tumour biology caused by genetic modification and/or administration of a therapy, which include radiotherapy and targeted agents.
Application of the 3Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	The plan is to identify new molecules underpinning the communication between tumour cells and their environment. This will involve scientific experiments using live mice, as it is not feasible to produce an adequate in vitro model that satisfactorily replicates the complex interactions between the different cells in the tumour.
2. Reduction How the use of minimum numbers of animals will be assured.	The number of mice required for each experiment has been calculated based on our extensive experience working with mice and with the advice from statisticians. Under most situations, the demonstration of similar effects in distinct animals (3 to 6) of identical phenotype is sufficient to establish the effect and rule out artefacts associated with biological variability. These experiments will be statistically designed to get as much information as possible with the least number of animals to be sacrificed.
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	The mouse constitutes an appropriate species because fundamental biological and pathological processes are similar or identical to those in other mammals, including humans. The mouse genome has been sequenced and can be manipulated to create genetically modified models. These models can be subsequently used to understand the function of genes. Animal suffering will be

measures to be taken to minimise welfare costs (harms) to the animals.	minimised by daily monitoring and by ensuring that staff are fully trained and assessed as competent. Where any signs of distress are present in an animal, then the animal will be euthanized immediately and any remaining mice of similar genotype observed closely for changes in their condition.