<table>
<tr><td>Project Title (max. 50 characters)</td><td>NKT cells and related immunity in health & disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cancer obesity NKT (cells) immunotherapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td><u>No</u></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td><u>Yes</u></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Tumour-bearing mice will be compared for an immune white blood cell subset, called Natural Killer T cells” (‘NKT’) cells and for tumour growth on standard and high fat diets to test the hypothesis that obesity-induced immune decline contributes to weakened anti-tumour responses. Interventions to reverse immune decline will be compared on tumour-bearing mice on standard and high fat diets to determine specifically whether reversing NKT defects aids anti-tumour responses.

NKT cells are a specialised immune cell type that can enhance anti-tumour immune responses. These cells can be activated by certain stimuli and other agents and vaccines, which may be able to stimulate anti-tumour immunity. This proposal will assess the ability of several such approaches to enhance immune responses to cancer, and determine how the responses to the best treatment can be further optimized. The goal will be to translate these results into a clinical trial taking into account cancer with and without obesity.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The purpose of the proposed research is evaluating novel immunotherapy for cancer and specifically, to determine if these approaches and strategies that manipulate immunity can eliminate tumours and if so, how. Modulating the immune system is a way to circumvent immune tolerance to tumour-associated antigens, a major clinical problem, which will be attempted to treat cancer in mice. The experimental design and findings emanating</td></tr>
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[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

	from this work will allow for a similar design in treating human cancer in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, up to 600 / year including breeding and experimental, x 5 years = 3000 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Progressive tumour growth will occur in un-protected mice which have not been protected by the therapies being tested (if that occurs) and in the control groups, but is likely less in the majority of the mice, since most are treated groups, based on previous results in other models. Tumour growth leads to moderate level distress, but death is not an endpoint and mice with progressively growing tumours will be euthanized once the largest tumour reaches a maximal size of 1 cm in any dimension. Also, regular monitoring and sacrifice of any ulcerating tumour-bearing or otherwise sick mice will minimise discomfort. Treatments will not induce or add to discomfort. All mice will be humanely euthanized before developing severe illness or by 1 year of age, if sooner.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Only live mice express the complete immune system and in transgenic tumour models, the tumour and immune system co-evolve, as in patients. Finally, one can only compare potential treatment strategies in mice, since there are no reliable tissue culture surrogates for immune anti-tumour responses to immunotherapies. Fortunately, however, the simplest feasible mammalian model, mice, provide all the advantages of similar physiology as well as well-characterised reagents (e.g. antibodies to cells and proteins) and tumour models are available and mechanistic insights from genetic models can be derived along with the ability to manipulate to determine how therapies work in a lower and non-endangered species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By comparing all treatments in parallel, the same controls will be used for all, thus reducing numbers from equal numbers of controls as treated mice to 40 % fewer mice in total. Published previous results in other tumour models and individual therapies indicates relatively small group sizes will be sufficient for clear results. As in clinical trials, once it becomes clear which approach(es) provide the best therapeutic effects, other approaches will be dropped and remaining control mice switched to the optimal means or sacrificed humanely.
3. Refinement Explain the choice of species	Mice have fundamentally similar immune systems to humans, including the NKT cells to be exploited

and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	in novel immunotherapeutic approaches. Thus there is no need for higher mammals, including primates, in this pre-clinical study. The tumour model to be employed is a relevant transgenic one (therefore close to human cancer) and directly relevant to the most likely clinical trials to follow. Previous such mouse studies have been accepted as sufficient for initiating a clinical trial of NKT cell reconstitution. This approach showed encouraging immune and tumour responses in a major medical centre setting, but is not practical at large scale in non-teaching hospital environments and further approaches which have been identified have wider and potentially greater therapeutic potential. Therefore, it is proposed to compare available NKT cell-based immunotherapy approaches together in the most efficient head to head comparison possible (based on limited tumour growth and immune studies, *not* survival), to provide pre-clinical data to support further clinical trials. In summary, these non-survival studies will employ our optimal protocols, maximising use of the 3 Rs.